SYNTHESIS OF REGIOSELECTIVERY SUBSTITUTED CELLULOSE DERIVATIVES AND THEIR PHYSICOCHEMICAL PROPERTIES(Dissertation_全文)

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CONTENTS

General Introduction

1. Significance of Studies on Regioselective Substitution of Hydroxyl Groups in Cellulose

Cellulose is a linear condensation polymer consisting of D-anhydroglucopyranose units (often abbreviated to anhydroglucose units or even to glucose units for convenience) jointly together by β-1,4-glucosidic bonds. It is thus a β-1,4-D-glucan. The $-CH_2OH$ and –OH groups, as well as the glycosidic bonds, are all equatorial with respect to the mean planes of the rings as illustrated in Figure 1.

The conventional numbering of the carbon atoms in the pyranose rings is shown in the left-handed non-reducing end-group in the same figure. The cellulose molecule is capable of forming both inter- and intramolecular hydrogen bonds. Four major crystal lattices have been reported for the celluloses that have different histories but contain no other substance. They have been designated cellulose I, II, III and IV. Each allomorph is identified by its unit cell constants rather than other criterion. Native celluloses form the crystal structure, cellulose I, which has been divided into two allomorphs of Iα and Iβ. Generally, regenerated celluloses[1] yield the more thermodynamically stable allomorph, cellulose II.

The three hydroxyl (OH) groups in an anhydroglucose unit of cellulose have different polarities. In particular, secondary OH at the C-2 position which is mostly affected by an anomeric carbon at the C-1 position has a stronger acidity than other hydroxyl groups such as secondary OH at the C-3 and primary OH at the C-6 position. While the secondary OH at the C-3 position tends favorably to form intramolecular hydrogen bonds with the adjacent glucose ring oxygen, the primary OH at the C-6 position is easy to form intermolecular hydrogen bonds. Accordingly variety of the inter- and intramolecular hydrogen bonding formation in cellulose has a strong influence on the physicochemical properties for the fibers and films.

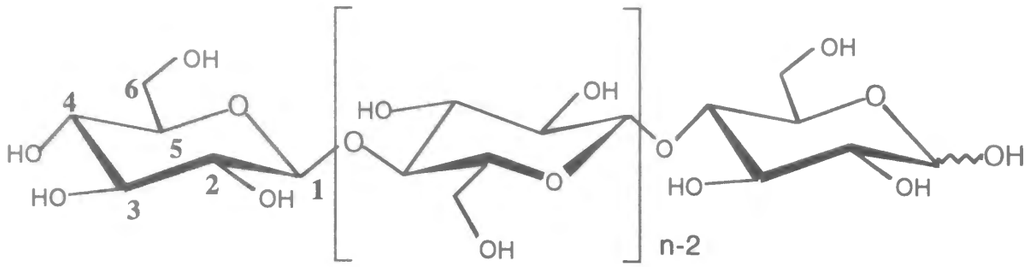


Figure 1. Chemical structure of cellulose.

I. OH : C-6-OH (Primary), C-2- and C-3-OH (Secondary)

II. Polarity (or Reactivity) in water: C-2-OH > C-6-OH > C-3-OH

III. Inter- and intramolecular hydrogen bonds

1. Crystal structure: $I(I_\alpha, I_\beta)$, II, III_I, III_{II}, IV_I, IV_{II}

Na-cellulose (alkali-cellulose)

2. Biosynthesis in plants and animals: cell wall cellulose formation

3. Solution properties : rigidity (or flexibility) and dimension of chains

4. Gel formation : sol ⇄ gel transition (thermotropic hydrogels)

5. Liquid crystallinity : lyotropic and thermotropic liquid crystals

6. Degradability : enzymatic

7. Adsorption : water and vapor

8. Physical properties

9. Biomass : energy conversion into alcohol

10. Derivatization : etherification, esterification, dehydration, etc.

Figure 2. A general idea on the correlation of basic characteristics for the three OH groups with the physicochemical properties.

Figure 2 shows a general idea on the correlation of basic characteristics for the three OH groups with the physicochemical properties. Cellulose exhibits really versatile properties from the transformation of crystal forms to the regiochemical difference of the reactivity for the chemical derivatization, which can be attributed to the polarity-difference for each hydroxyl group in an anhydroglucose unit as mentioned above.

Chemical processing of cellulose proceeds during its whole course or at the initial stage in a heterogeneous reaction medium. Rates and final degrees of conversion in these heterogeneous reactions depend strongly on the availability of the OH groups in the anhydroglucose units, the so-called accessibility of the OH groups. From the sight of cellulose structure, the accessibility depends largely on the available inner surface, and also on the supermolecular order and fibriller architecture. From a chemical point of view, a nonuniformity of functionalization along the single β-1,4-D-glucan chain and within an anhydroglucose unit has a priority to be taken into account. The nonuniformity along the molecular chain depends on the difference in accessibility of reagents between the ordered and amorphous regions in cellulose. Concerning the substitution pattern of the OH groups within an anhydroglucose unit, the OH group at the C-3 position is generally not favored to be reacted in heterogeneous reactions. As already mentioned, the OH at the C-2 position is more acidic than that at the C-6 position, and the reactivity of the three OH groups is in the order of OH-3<OH-6<OH-2 in aqueous systems[2,3]. The order of reactivitiy in a non-aqueous system is reported to be OH-3<OH-2<OH-6[4].

It is thus very important to prepare the regioselectively substituted cellulose derivatives from the two following points.

i) A new design of functionalized materials from cellulose

The functional properties of cellulose derivatives strongly depend on the total degree of substitution (DS), molar substitution (MS), the distribution of substituents within the anhydroglucose units and along the cellulose chain. In addition, they depend on the distribution of DS and MS. The distribution within the anhydroglucose

unit arises due to the difference in the polarity among the three OH groups as described above. Therefore the regioselective derivatization of cellulose is very important for its heigher functionalization[5,6]. It is also important to control the processing and end-use properties by the pattern of functionalization and to develop advanced cellulose materials having well-ordered supramolecular structures. The new fields of application will be liquid crystalline polymers[7,8], selective membranes, multilayered assemblies[9,10], sensor matrices, recognition devices[11], and bioactive materials[12, 13].

ii) As model compounds for investigating the physicochemical properties of cellulose and its derivatives

Regioselectively substituted cellulose derivatives are considered as cellulosic materials which may have controlled inter- or intramolecular hydrogen bonds. They may exhibit different properties which have not been seen so far. Another advantage of studying regioselectively substituted cellulose derivatives is to make it easier to understand the relationship between the physicochemical properties and the hydrogen bonding formation of cellulose. In order to produce functional derivatives with well-defined structures, it is also important to elucidate the characteristics of inter- and intramolecular hydrogen bonds in cellulose. The regioselectively substituted cellulose derivatives are very useful as model compounds for this purpose.

2. Regioselectively Substituted Cellulose Derivatives

Here, the term "regioselectivity" in cellulose is defined as an exclusively or significantly preferential reaction at one or two of the three different OH groups at the C-2, C-3 and C-6 positions of the anhydroglucose unit. The energy profile of a typical regioselective reaction demonstrates that steric hindrance by bulky groups and entropic acceleration caused by hydrogen bonds and/or electronic promotion (e.g., electronically withdrawn substituents) are essential to differentiate among the free

enthalpies of activation as well as of rate constants and concentrations of the products.

For the synthesis of cellulose derivatives with well-defined structures, the five following methods have been developed[14]:

(i) the polymer-analogous reactions of cellulose. This is a most convenient derivatization pathway. In this pathway, two kinds of methods have been developed. Firstly, the regioselective partial derivatization of cellulose and subsequent reactions of the free OH groups followed by the elimination of the primaty groups. This thesis is mainly based on this synthesis method. Secondly, the complete derivatization of cellulose followed by a site-selective (regioselective) reaction of the introduced groups and by the elimination of the primary groups;

(ii) the enzymatically catalyzed regioselective modification, e.g. esterification or oxidation of the cellulose as experimentally realized by the lipase-catalyzed acylation of oligosaccharides[15-17];

(iii) the enzyme catalyzed polymerization of glucose derivatives to give macromolecules with a preset structure. This method is of still rather visionary character[18];

(iv) the stepwise construction of sequential cellulose-based macromolecules from glucose derivatives. This is analogous to protein synthesis, which is a very tedious method to produce polymer chains of a defined functionalization pattern not only along the macromolecules but also within the single anhydroglucose unit.

(v) the cationic ring-opening polymerization of orthoesters from glucose derivatives[19]. This method could be applied to the synthesis of not only regioselectively substituted cellulose derivatives but also block co-polymers of polysaccharides.

To obtain the regioselectively substituted cellulose derivatives by the method employed in this thesis, the primary OH groups at the C-6 position are usually blocked by bulky protective groups. The two major protective groups for the OH groups at the C-6 position which provide for subsequent selective functionalization of

the remaining secondary OH groups have been widely used; triphenylmethyl (trityl) group[20-26] or its derivative[27,28], and thexyldimethylchlorosilane (TDMS) group[29,30].

Regarding regioselectivity of reactions within the anhydroglucose unit, frequently high regioselectivity, e.g. an explicit functionalization at the C-6 and C-3 positions, is required to arrive at unexpected properties of the compounds. Even a moderate preference of a site of substitution often suffices to modify physicochemical properties, depending on intermolecular interaction with a solvent or with living matter, as demonstrated recently by Klemm *et al.*[31]

Besides regioselective derivatives with free OH groups, fully substituted derivatives (DS=3) are also of interest with regard to their material properties. However, they still pose experimental problems in the synthesis.

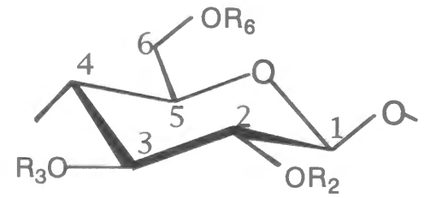
3. Outline of This Thesis

This thesis consists of three parts: Part I (Chapters 1-3) deals with the preparation of regioselectively substituted cellulose ethers, i.e., 2,3-di-*O*-alkylcelluoses, 6-*O*-alkylcelluoses, tri-*O*-alkylcelluoses. Part II (Chapters 4-7) concerns the hydrogen bond formation in cellulose derivatives prepared according to the methods developed in Chapters 1-3, mainly using FT-IR, fluorescence and solid state NMR spectroscopies. Part III describes the results on the enzymatic degradation of regioselectively methylated cellulose ethers as an example of the applications of regioselectively substituted cellulose derivatives.

Since the OH groups form controlled inter- and intramolecular hydrogen bonds in regioselectively substituted cellulose derivatives, such derivatives are considered to be very useful as cellulose model compounds to investigate the relationships between the formation of hydrogen bonds and physicochemical properties of cellulose and its derivatives. The problem is whether or not the ring conformation of the anhydroglucose unit still remains unchanged even by the introduction of the substituents to OH groups of cellulose. Methyl group is the smallest substituent and

non-polar. It is expected that the conformation of the anhydroglucose rings is little affectd by the introduction of methyl groups into cellulose. In fact, later the author and Sawatari have proved that the above-mentioned consideration is correct[32]. In any case, regioselectively methylated derivatives are useful as cellulose model compounds to controll the formatin of hydrogen bonds in cellulose.

In Chapters 1 to 3, attempts are made to synthesize regioselectively substituted cellulose ethers; 2,3-di-*O*-, 6-*O*- and tri-*O*-substituted cellulose derivatives[22,24,33] shown in Figure 3. Facile preparation methods for these derivatives will be reported in details.



	R_2	R_3	R_6
Cellulose	H	H	H
2,3-Di-*O*-	R	R	H
6-*O*-	H	H	R
Tri-*O*-	R	R	R

R: Alkyl, Allyl, Benzyl, and
Trityl (6-*O*-substituted only)

Figure 3. The chemical structures of regioselectively substituted cellulose derivatives synthesized in Chapters 1-3.

In Chapter 4, the noncrystalline films of regioselectively substituted cellulose ethers, namely, 2,3-di-*O*- and 6-*O*-methylcelluloses, were prepared and the formation of hydrogen bonds in these regioselectively substituted cellulose derivatives are investigated using FT-IR and CP/MAS ^{13}C-NMR spectroscopies[34]. As shown in Figure 4, various types of inter- and intramolecular hydrogen bonds are considered to

exist in cellulose and cellulose derivatives prepared. The spectra are compared with those of the starting cellulose, and the results will be discussed in terms of hydrogen bonds formed.

It is well known that the formation of inter- and intramolecular hydrogen bonds in cellulose and its derivatives has a strong influence on their physical properties. However, only a few reports[35-37] have dealt directly with the above point of view, although many papers[38,39] have referred indirectly to the relationship between the hydrogen bonding formation and the physicochemical properties. Chapters from 5 to 7 attempt to clarify the relationship between the hydrogen bonding formation and the physicochemical properties using films, liquid crystals and gels [40-43], which have been prepared from regioselectively substituted *O*-alkylcelluloses.

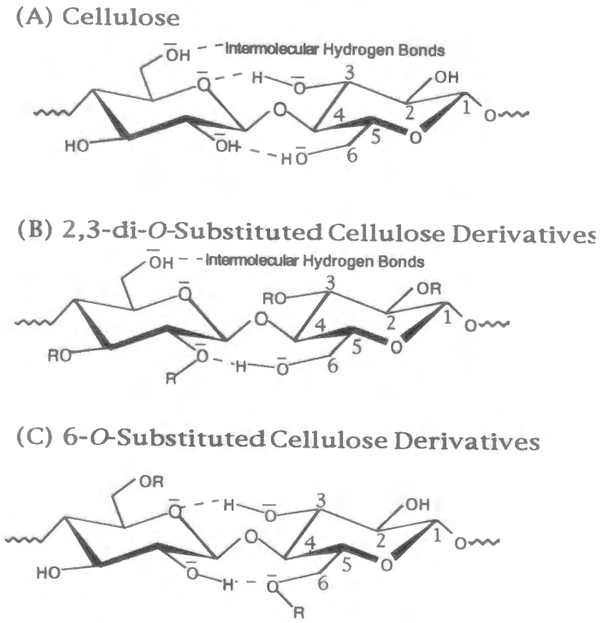


Figure 4. Schematic representation of possible hydrogen bonds involved in cellobiose units of regioselectively substituted cellulose derivatives prepared.

These investigations will be performed mainly using some sophisticated tools such as FT-IR, fluorescence and CP/MAS NMR spectroscopies. Especially, throughout these studies, the author wants to elucidate how the intramolecular hydrogen bonds at the C-3 position of cellulose and cellulose derivatives contribute to their physicochemical properties.

In Chapter 8, the enzymatic degradability of the regioselectively substituted *O*-methylcelluloses are investigated[44]. The emphasis is put on the influence of regioselective substitution in *O*-methylcelluloses on their enzymatic hydrolyses, which may provide an important information for further developments of biodegradable materials from cellulose derivatives.

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Part I

Preparation of Regioselectively Substituted Cellulose Ethers

Chapter 1

Facile Method for the Preparation of Tri-*O*-alkylcelluloses

ABSTRACT: A simple way to prepare tri-*O*-alkylcelluloses from cellulose acetate (CA) in dimethylsulfoxide (DMSO) using the appropriate alkyl iodide and NaOH, has been developed. In this procedure, the addition of a small amount of water to the DMSO solution improved the efficiency of etherification. The substitution pattern of *O*-methylcellulose (MC) was investigated to determine the effect of added water on the relative reactivities of the glucose ring substituents. This etherification method can be applied to alkylate other polymers with acetate-functional groups that are soluble in DMSO.

INTRODUCTION

Alkylcellulose ethers are an important class of industrial polymers. They are prepared commercially through the heterogeneous reaction of aqueous alkalicelluloses with alkyl halides or epoxides. Under these reaction conditions, it is difficult to prepare alkylcellulose ethers with high degrees of alkyl group substitution (DS) for the following reasons:

1. Water can participate in undesired side reactions.
2. Heterogeneous reaction conditions may prevent the reagents from penetrating the starting material.
3. The alkylating reagents have limited solubility in water.

Isogai *et al.*[1,2] proposed a method for the complete alkylation of cellulose in the nonaqueous cellulose solvent SO_2-diethylamine(DEA)-dimethylsulfoxide(DMSO).

Modifications of the Hakomori method[3] have been reported[4-6] for the preparation of *O*-methylcelluloses (MC), but some chain degradation and side reactions occurred. Permethylation of sugars using solid NaOH in DMSO was reported by Ciucanu and Kerek[7]. Recently, Kondo and coworkers[8] have succeeded in the quantitative preparation method of tri-*O*-allylcellulose from cellulose acetate (CA) in DMSO. This chapter reports an improved procedure that results in the facile preparation of tri-*O*-alkylcelluloses. Interestingly, water, which normally reduces the efficiency of etherification reactions, has been observed to play an important role in this process.

EXPERIMENTAL

Materials

Commercial cellulose acetate samples from cotton linters were provided by Daicel Chemical Ind. Co., Ltd. (DS 1.75, 2.24 and 2.39). The samples were dried at 105 °C for 3 hours. The DMSO was dehydrated over type 3A molecular sieves. Reagent-grade solvents and alkylating agents (Aldrich Gold Label) were used without further purification. Powdered sodium hydroxide was pulverized by grinding NaOH pellets in a domestic coffee mill.

Preparation Methods

All starting solutions were prepared as follows: 1g cellulose acetate was completely dissolved in 60 mL DMSO at 60°C for 1 hour with constant stirring. The amounts of powdered NaOH and alkylating agents were adjusted according to the number of anhydroglucose units present in 1g of the cellulose acetate (Table 1-1).

The methylation step was investigated using four different reaction conditions to determine the optinum conditions for the preparation of tri-*O*-alkylcelluloses from CA.

Series A *O*-methylcellulose samples (MC-A) were prepared according to Scheme 1, as reported previously[1].

Series B samples were prepared as for series A, but with reaction times at 60°C increased from 2 - 21 hours.

To prepare series C samples, iodomethane (CH_3I) was added dropwise at four different times during the reaction. The first portion comprised two thirds of the total amount of CH_3I. The three remaining portions comprised one ninth of the total, as shown in Scheme 2.

Series D samples were prepared as for series C, but 1 mL of distilled water was added to the starting CA-DMSO solution.

Table 1-1. Preparative conditions of *O*-methylcelluloses (MCs) from cellulose acetate (CA) with DS 2.24..

Sample (MC-X(N,M))[a]	NaOH added (g/gCA)	CH_3I added (mL/gCA)
MC-X(5.0,5.0)	2.3	3.7
MC-X(7.5,7.5)	3.5	5.6
MC-X(10.0,10.0)	4.7	7.4
MC-X(12.5,12.5)	5.9	9.3
MC-X(15.0,15.0)	7.0	11.2

a) X indicates the preparative method, either A,B,C or D depending on sample series. N and M are the number of moles of NaOH and alkyl iodide, respectively, per three hydroxyl groups (substituted and unsubstituted) in each anhydroglucose unit in the CA sample.

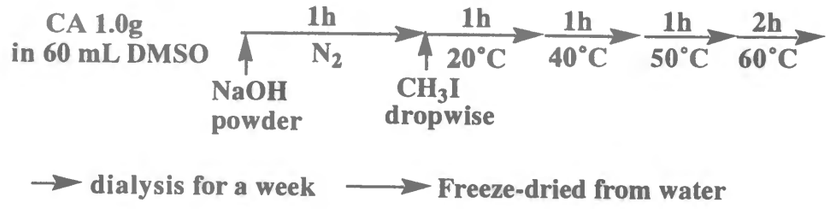
For example, the amount of NaOH per gram CA is

(1.0 / MW) x 3 x N x 40 (g),

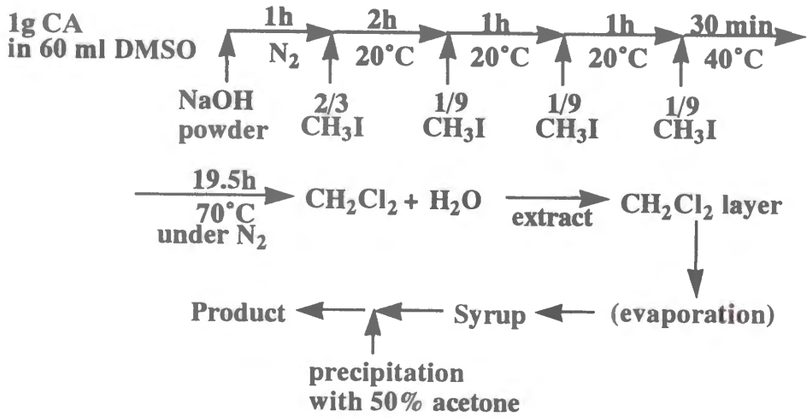
where

MW (molecular weight of 1 acetylglucose unit) =162 + 42 x DS.

The amount of CH_3I per gram CA :(1.0/MW) x 3 x N x 141.94 / 2.28 (mL).



Scheme 1. Procedure for preparation of series A of MC samples.

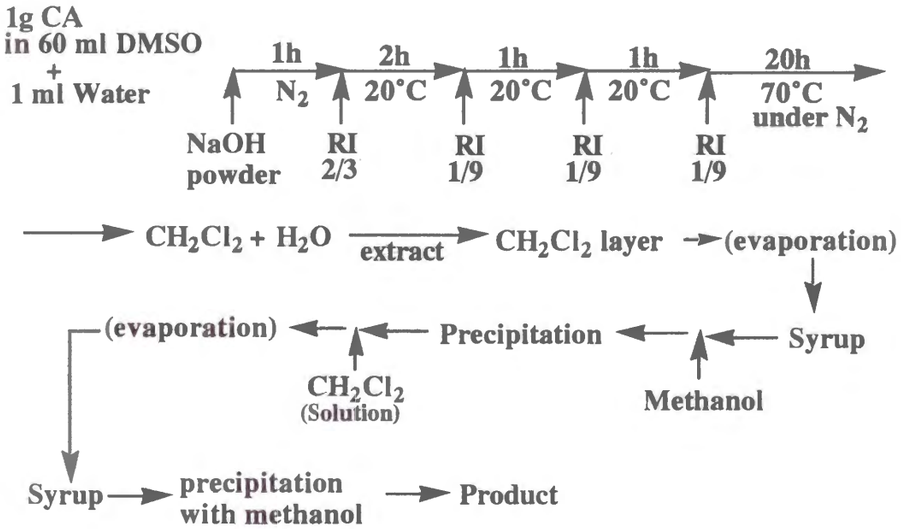


Scheme 2. Procedure for preparation of series C of MC samples.

Complete Alkylation of Cellulose

The series D method (Scheme 3) was applied to the preparation of other cellulose ethers. Typically, 5.86 g of powdered NaOH was dispersed in a CA-DMSO solution containing 1 mL of water. Following 1 hour stirring under a nitrgen atmosphere, two thirds of the total amount of the alkyl iodide (RI) was added dropwise to the solution. At 2, 3, and 4 hours after the first addition of RI, one third of the remaining RI was added dropwise, respectively. Following the last addition of RI to the solution, the temperature was raised to 70°C and kept at this temperature for 20 hours under a

nitrogen atmosphere. The reaction mixture was cooled to room temperature, and 100 mL of both water and methylene chloride was added. The methylene chloride layer was separated, washed with water and evaporated under reduced pressure at 40°C until a syrup remained. The tri-*O*-alkylcellulose was precipitated from the syrup by the addition of 100 mL of methanol (50% acetone for the methylation product). Following filtration, the precipitate was dissolved in 100 mL of methylene chloride and reprecipitated according to the same procedure. The filtered product was air-dried followed by drying under vacuum at 60°C.



Scheme 3. Procedure for complete alkylation of cellulose with alkyl iodide (RI).

Analyses

The distribution of methyl groups in the MC samples was determined by acid hydrolysis and analysis of the components as partially methylated alditol acetates by gas-liquid chromatography (GLC)[9]. MC (50 mg) was dissolved in 15 mL of 3%

sulfuric acid and was hydrolyzed at 120°C for 1 hour. The hydrolyzates were neutralized at pH 5.5 with barium carbonate, and the precipitated barium sulfate was removed by centrifugation. The partially methylated glucoses thus obtained were reduced with $NaBH_4$ and then acetylated. The partially methylated alditol acetates were analyzed by a Hewlett-Packard Model 5890A gas-liquid chromatograph equipped with a Hewlett-Packard Model 3392A integrator, a flame-ionization detector, and a J. and W. Scientific DB-1 fused-silica capillary column (0.25 mm x 30 m; film thickness 0.25 μm). The temperature was increased from 190°C to 215°C at 1°C / min.

IR spectra were obtained with a Mattson Cygnus Fourier transform infrared (FT-IR) spectrophotometer with photoacoustic detection. ^{1}H- and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained from a Varian XL 200 spectrometer at frequencies of 200 MHz and 50.4 MHz, respectively. Deuterated chloroform ($CDCl_3$) was used as the solvent at 30°C. Spectra were referenced relative to $CHCl_3$ for ^{1}H-NMR and $CDCl_3$ for ^{13}C-NMR measurements.

Gel permeation chromatograms in chloroform and tetrahydrofuran were obtained with a Waters ALC/GPC 244 system and 10^5, 10^4, 10^3, 500 and 100 Å μ-Styragel colums in series. The column set was calibrated with poly(styrene) standards.

RESULTS AND DISCUSSION

Preparation of MC

Table 1-2 shows the results of GLC analyses of the substituent distribution (Xn (n=2, 3 and 6)) for the MC samples. X_2, X_3 and X_6 were calculated from molar ratios of glucitol (S_0) and its mono-, di- and tri-derivatives (S_2-S_{236}) (Figure 1-1) which were derived from the hydrolysates of the polymers as determined by GLC:

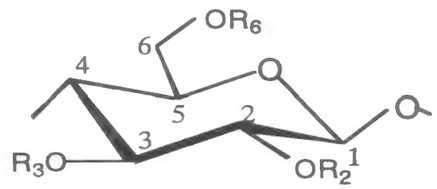
$$X_2 = S_2 + S_{23} + S_{26} + S_{236}$$

$$X_3 = S_3 + S_{23} + S_{36} + S_{236}$$

$$X_6 = S_6 + S_{26} + S_{36} + S_{236}$$

$$DS = X_2 + X_3 + X_6$$

These values (Xn) indicate the reactivities of each hydroxy group, as well as the distribution of substituents at OH in each position (C-2, C-3 and C-6) of the ring carbons. The DS represents the sum of X_2, X_3 and X_6.



	R_2	R_3	R_6
(1)	H	H	H
(2)	Alkyl	H	H
(3)	H	Alkyl	H
(4)	H	H	Alkyl
(5)	Alkyl	Alkyl	H
(6)	Alkyl	H	Alkyl
(7)	H	Alkyl	Alkyl
(8)	Alkyl	Alkyl	Alkyl

Figure 1-1. The structure of 8 glucoside residues of the products.

Series A did not yield tri-*O*-methylcellulose. Complete methylation was not achieved despite varying the amount of reagents, the DS of starting materials, and the reaction time in the procedure of Scheme 1 (Samples MC-A and MC-B in Table 1-2). The methyl group DS reached a limit after addition of 7.5 mol reagent per mol of substituted and unsubstituted hydroxy groups for two samples. From values of Xn, it was determined that the relative reactivity of hydroxy groups (OH-2, OH-3 and OH-6, respectively) at the two (C-2), three (C-3) and six (C-6) positions of the anhydroglucose unit was in general OH-2 > OH-6 > OH-3 in this system. This order of reactivities is the same as that reported for MC prepared from alkalicelluloses in an

Table1-2. Distribution of methyl groups in *O*- methylcellulose samples.[a)]

Samples	DS[b]	Molar Ratios (%)[c]								DS in Each Position		
		S_0	S_2	S_3	S_6	S_{23}	S_{36}	S_{26}	S_{236}	X_2[d]	X_3	X_6
From DS 2.24												
MC-A(5.0,5.0)	1.85	10.6	16.3	8.5	2.6	13.7	3.7	11.2	33.5	0.75	0.59	0.51
MC-A(7.5,7.5)	2.80	0	0.6	0	0	7.4	2.0	9.6	80.4	0.98	0.90	0.92
MC-A(10.0,10.0)	2.73	0	1.0	0.2	0.2	8.6	2.2	12.5	75.3	0.97	0.86	0.90
MC-A(12.5,12.5)	2.70	0.4	1.3	0	0.4	7.1	1.7	16.9	72.3	0.98	0.81	0.91
From DS 2.39												
MC-A(5.0,5.0)	2.64	0.7	3.1	0.9	0.5	9.4	1.7	11.5	72.2	0.96	0.84	0.84
MC-A(7.5,7.5)	2.81	0	0.4	0	0	6.4	2.1	9.3	81.8	0.98	0.90	0.93
MC-A(10.0,10.0)	2.77	0	1.2	0.2	0	9.8	1.2	9.6	78.0	0.99	0.89	0.89
MC-A(15.0,15.0)	2.77	0	0	0.7	0	5.3	1.1	15.2	77.7	0.98	0.85	0.94
From DS 2.24												
MC-B(10.0,10.0)	2.71	0	1.5	0.3	0.2	9.2	2.6	13.7	72.4	0.97	0.85	0.89
MC-C(12.5,10.0)	2.94	0	0	0	0	3.6	0.8	2.6	93.0	0.99	0.97	0.96
MC-D(10.0,10.0)	2.89	0	0.2	0.2	0.1	4.8	1.4	3.5	89.9	0.98	0.96	0.95
MC-D(12.5,10.0)	3.00	0	0	0	0	0	0	0	100.0	1.00	1.00	1.00

[a] Nomenclature of samples is given in Table 1-1.

[b] $DS=X_2+X_3+X_6$

[c] Calculated from yields of glucitol (S_0) and its partially methylated derivatives (S_2-S_{236}) determined by GLC.
For example, S_2 means the molar ratio of 2-methylglucitol acetate.

[d] X_n is the ratio of glucitol derivatives substituted at the OH of carbon n (n = 2,3,and 6) position.
For example, $X_2 = (S_2+S_{23}+S_{26}+S_{236})/100$

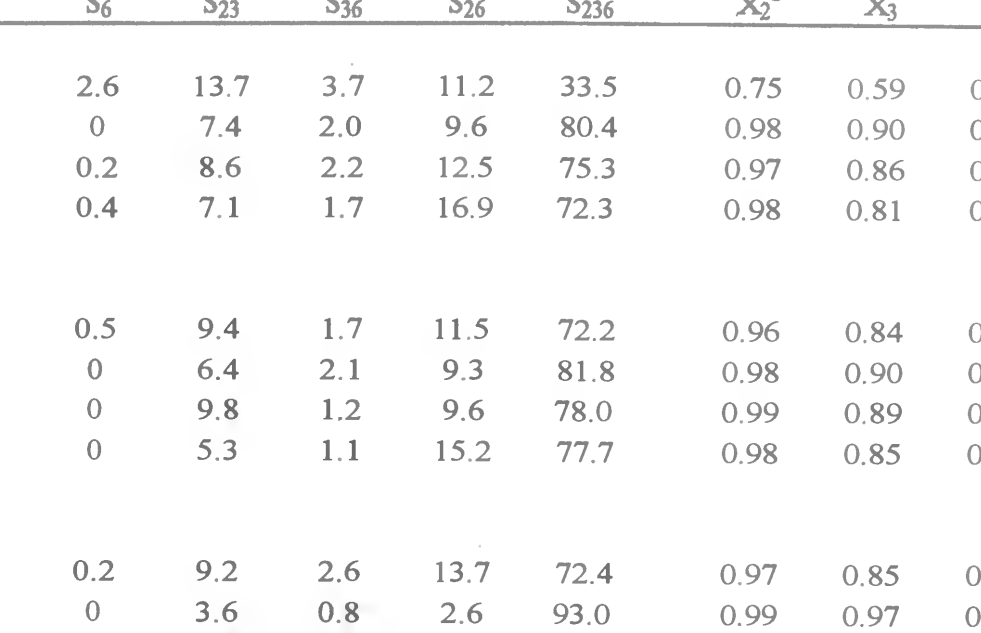


Figure 1-2. IR spectra for the *O*-methylcelluloses (in series C and D).
(1) CA (DS, 2.24) starting material. (2) MC-C(10.0,10.0)
(3) MC-C(12.5,10.0) (4) MC-D(10.0,7.5) (5) MC-D(10.0,10.0)
(6) MC-D(12.5,10.0) (tri-*O*-methycellulose).
Nomenclature of samples is described in Table 1-1.

aqueous solvent system[10,11], but differs from the order (OH-6 > OH-2 > OH-3) observed in a non-aqueous system[11].

Figure 1-2 shows IR spectra of MC prepared by stepwise addition of reagents (series C and D). MC-C(10.0, 10.0) (curve 2 in Figure 1-2) shows complete methylation of the free hydroxy groups in CA. Following methylation, the number of acetyl groups remaining from the original CA (curve 1 in Figure 1-2) is very small. The addition of an excess amount of powdered NaOH to the amount of CH_3I, resulted in a reduction in the number of acetyl groups (curve 3 in Figure 1-2). This MC-C(12.5,10.0) sample did not have hydroxy groups and the remaining acetyl groups were mainly in the C-6 and C-3 positions [MC-C(12.5,10.0) in Table 1-2]. Thus, acetyl groups in C-6 and C-3 positions are most resistant to saponification in this system.

These etherification reaction conditions can be considered heterogeneous. This is due to the solid state of the powdered NaOH used as a base in the CA-DMSO solutions. The addition of a small amount of water in series D may solubilize the solid NaOH. Following the addition of 1 mL of water to the CA-DMSO solution, the solution remained homogeneous. Varying the quantities of reagents led to the optimum conditions for the preparation of tri-*O*-methylcellulose shown in the last entry of Table 1-2, with 1 mL of distilled water and 12.5 mol/mol and 10 mol/mol of powdered NaOH and iodomethane, respectively. (In fact, methylation does not require heating at 70°C for prolonged periods as was the case for the other alkylations.)

The ^{13}C-NMR chemical shifts for these samples are identical to those reported previously; C-1(103.0 ppm), C-2(83.7 ppm), C-3(85.2 ppm), C-4(77.6 ppm), C-5(74.7 ppm), C-6(70.3 ppm) and carbons of methyl substituents(59.0, 60.3 and 60.5 ppm)[1]. The other CA samples with DS's of 2.39 and 1.75 also yielded tri-*O*-methylcellulose when subjected to these conditions. Thus, the NMR and IR evidences confirm the formation of completely substituted MC, independent of the DS of the cellulose acetate starting material.

Influence of Water and Reagent Quantity on Reaction Efficiency

The addition of a small amount of water improves the efficiency of the methylation reaction. According to the results in the Table 1-3, an increase in the amount of water

Table 1-3. Distribution of methyl groups in MCs prepared by changing the amount of water and CH_3I

Sample	DS	Molar Ratios (%)						DS in Each Position			
		S_0	S_2	S_3	S_6	S_{23}	S_{36}	S_{26}	S_{236}	X_2	X_3	X_6
MC-D(12.5,10.0)[a]												
1. +10mL H_2O	0.91	55.7	6.1	2.0	8.6	3.9	1.3	3.3	19.2	0.33	0.26	0.32
2. +5mL H_2O	1.28	35.8	5.9	1.7	18.2	5.1	3.1	5.1	25.1	0.41	0.35	0.52
3. +2mL H_2O	2.99	0	0	0	0	0.5	0.4	1.3	97.8	1.00	0.99	1.00
4. +1mL H_2O	3.00	0	0	0	0	0	0	0	100.0	1.00	1.00	1.00
With 1 mL water addition												
5. MC-D(12.5,5.0)	2.97	0	0	0	0	0.9	0.7	1.0	97.5	0.99	0.99	0.99
6. MC-D(12.5,2.5)	2.98	0	0	0	0	1.2	0.5	1.1	97.2	1.00	0.99	0.99
7. MC-D(12.5,1.0)	2.86	0.6	0	0	4.0	1.4	1.4	1.9	90.7	0.94	0.94	0.98

[a] Nomenclature of samples is given in Table 1-1.

results in a decrease in the DS. The addition of water may result in the dissolution of NaOH in the DMSO solvent. The addition of powdered NaOH to a CA-DMSO solution without water resulted in the precipitation of polymer. However, no precipitate formedon addition of powdered NaOH when 1 mL of water was added to the CA-DMSO. The more homogeneous solution observed in the presence of water may facilitate saponification of the actyl groups in the C-6 position.

Interestingly, in the cases where 5 and 10 mL water were added, there were high molar ratios of S_0 and S_{236} corresponding to unsubstituted and completely substituted glucose units, respectively (nos.1 and 2 in Table 1-3). This suggests that these *O*-methylcelluloses are blocky in nature.

The quantity of reagents had little effect on the methylation efficiency. The addition of 2.5 mol CH_3I/ mol yielded the almost totally methylated cellulose and 10.0 mol CH_3I/ mol addition was sufficient to give tri-*O*-methylcellulose (nos.4, 5, and 6 in Table 1-3).

The reactivity order of hydroxy groups in series D was OH-6≥OH-2 ≥OH-3, very similar to that proposed for the SO_2-DEA-DMSO[12] even though a trace of water was present in the solution.

Application to the Complete Alkylation of Cellulose

As shown in Table 1-4, tri-*O*-alkylcelluloses from methyl to n-decyl were obtained in high yields without significant depolymerization. The derivatives with alkyl substituents from methyl to n-pentyl were isolated as white solids; tri-*O*-n-hexyl, tri-*O*-n-hepty, tri-*O*-n-octyl, tri-*O*-n-nonyl and tri-*O*-n-decylcelluloses were isolated as sticky solids.

The IR spectra for tri-*O*-methyl, tri-*O*-propyl, tri-*O*-n-pentyl and tri-*O*-n-octylcelluloses are shown in Figure 1-3. These show no hydroxyl absorption band around 3400 cm^{-1}. The absorptions around 2800-3000 cm^{-1} due to C-H streching vibratuions and 1460 and 1370 cm^{-1} due to C-H bending vibrations increased with an

Table1-4. Cellulose ethers (Series D) prepared with water[a], powdered NaOH[b] and alkyl iodide in the CA[c]-DMSO solution.

No.	Reagent	Amount (mL) (1st portion,others)	DS	yield (%)	Solvent for Precipitation	M_w[d] ($\times 10^5$)
1	CH_3I	4.9,0.8(3times)≧	3.0	94	50% Acetone	0.92
2	CH_3CH_2I	6.3,1.1(3times)	3.0	99	Methanol	0.89
3	$CH_3(CH_2)_2I$	7.3,1.4(3times)	3.0	88	Methanol	0.85
4	$CH_3(CH_2)_3I$	9.0,1.5(3times)	3.0	80	Methanol	0.85
5	$CH_3(CH_2)_4I$	10.4,1.7(3times)	3.0	89	Methanol	-
6	$CH_3(CH_2)_5I$	11.8,1.9(3times)	3.0	60	Methanol	-
7	$CH_3(CH_2)_6I$	13.0,2.2(3times)	3.0	62	Methanol	-
8	$CH_3(CH_2)_7I$	14.4,2.4(3times)	3.0	60	Methanol	-
9	$CH_3(CH_2)_8I$	16.5,2.8(3times)	3.0	73	Methanol	-
10	$CH_3(CH_2)_9I$	16.8,2.8(3times)	3.0	60	Methanol	-

[a]The amount of water was 1.0 mL.

[b]The amount of powdered NaOH was 5.86 g.

[c]CA ; cellulose acetate with DS of 224 and M_w of 1.09×10^5.

[d]Approximate M_w ; values are for poly(atyrene)chains whoes GPC elusion curves correspond to the cellulose ethers.

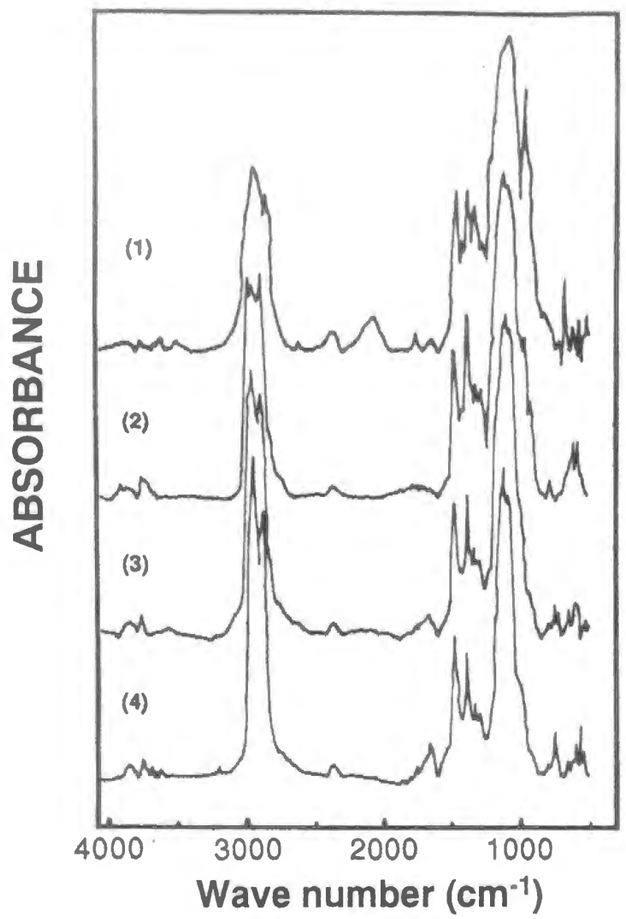


Figure 1-3. IR spectra of tri-*O*-alkylcelluloses.
(1) Tri-*O*-(methyl)cellulose, (2) Tri-*O*-propylcellulose,
(3) Tri-*O*-n-pentylcellulose, and (4) Tri-*O*-n-octylcellulose.

increase in the chain length of the n-alkyl substituents. ^{13}C-NMR chemical shifts of these derivatives were in agreement with published data[1]. The observed absence of splitting for each of the ring carbons indicates complete substitution of the neighboring

carbons[13]. No side reactions such as oxidation were indicated in Figure 1-3. The absence of splitting for each of the ring carbons indicates complete substitution of the neighbouring carbons[13]. No evidence of side reactions such as oxidations was detected by IR or NMR spectroscopies.

Preliminary results showed that this method was also effective for the complete methylation and ethylation of poly (viny alchohol) (Mw 78000, Polyscience Inc.) and poly (vinyl acetate) (Mw 500000, Polyscience Inc.) in one step. IR analysis confirmed the absence of hydroxyl and acetyl bands in the products. Thus, the method can be applied to other polymers that are soluble in DMSO.

CONCLUSIONS

1. Tri-*O*-alkylcelluloses were prepared without significant depolymerization by adding powdered NaOH and alkyl iodide to a solution of cellulose acetate in DMSO containing a small amount of water.
2. In this procedure, the addition of a small amount of water improved the efficiency of alkylation of the cellulose acetate, presumably by facilitating hydrolysis. Addition of larger amounts of water resulted in incomplete alkylation.
3. The order of the reactivity of the acetylated starting materials was C-2 > C-6 > C-3 in the water-free DMSO system (series A), and C-6 ≧ C-2 ≧ C-3 in the DMSO-water system (series D).
4. This novel method can also be applied to the alkylation of other macromolecules soluble in DMSO.

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Chapter 2

Preparation of *O*-Methyl and *O*-Ethylcelluloses Having Controlled Distribution of Substituents

ABSTRACT: *O*-Methyl and *O*-ethylcelluloses having controlled distribution of substituents were prepared by alkylation of 6-*O*-triphenylmethyl ("trityl") cellulose in dimethylsulfoxide (DMSO) and subsequent detritylation of the alkylated products. Water-free alkylation reactions failed to give complete substitution of the hydroxyl groups at both the C-2 and C-3 positions. The addition of a small amount of water to the tritylcellulose solution in DMSO improved the efficiency of the alkylations, yielding 2,3-di-*O*-substituted-6-*O*-tritylcellulose, which in turn gave the 2,3-di-*O*-alkylcelluloses on detritylation with HCl gas. Repeated further alkylation of the 2,3-di-*O*-alkylcelluloses in DMSO furnished products with high degrees of substitution at the C-6 position. The distribution of substituents in the alkyl cellulose ethers was determined by acid hydrolysis, reduction, and gas-chromatographic separation of the partially alkylated alditol acetates. The distribution of methyl and ethyl groups in the polymers prepared by repeated alkylation systematically changed with each alkylation step.

INTRODUCTION

O-Alkylcelluloses such as *O*-methylcellulose and *O*-ethylcellulose have received considerable attention industrially. The distribution of alkyl groups in the anhydroglucose units as well as along the chain is considered to influence strongly phisical properties such as solubility, crystalization, gel formation, liquid crystal

formation and resistance to enzymatic degradation. The precise determination and control of the distribution of substituents in the synthesis of cellulose ethers are important in clarifying the correlation between the physicochemical properties of these derivatives and their chemical structure.

The determination of distribution of substituents in alkylcellulose derivatives has been extensively examined by paper chromatography[1,2], GLC[3-6] and ^{13}C-NMR.[7-11] techniques. However, only a few reports[12-14] have related physical properties to the distribution of substituents of cellulose ethers and esters. This lack of correlative effort is due to the difficulty of preparing alkylcellulose derivatives having controlled distribution of substituents.

In the previous chapter[15], a facile method for the preparation of tri-*O*-alkylcelluloses has been reported. It was found that a small amount of water added to the reaction mixture improved the reactivity of the hydroxyl groups, especially at the C-3 position. In this chapter, *O*-triphenylmethyl("trityl")cellulose in which the C-6 hydroxyl is regioselectively substituted[16,17], was used as a starting material, since this group can be easily removed by exposing the *O*-tritylcellulose derivatives to acidic conditions[18-20]. This allows the preparation of cellulose derivatives that are regioselectively substituted at C-2 and C-3 position[20,21].

The beneficial effect of added water on the preparation of cellulose triethers also facilitated the preparation of *O*-methyl and *O*-ethylcelluloses that were completely substituted at the C-2 and C-3 positions. These products have been characterized by hydrolysis, reduction, and GLC of resulting alditol actates, FTIR and ^{13}C-NMR. These polymers would appear to be ideal samples for determining a correlation between physochemical properties and the distribution of substituents in *O*-alkylcellulose derivatives.

EXRERIMENTAL

Materials

Commercial cellulose acetate samples were provided by Eastman (acetyl content 39.9 %, ASTM viscosity 27). The samples were completely deacetylated in a 15 % aqueous solution of ammonium hydroxide for 14 days at room temperature. The dimethylsulfoxide (DMSO) and pyridine were dehydrated over type 3A molecular sieves. Reagent grade solvents and reagents (Aldrich Gold Label) were used without further purification. Powdered sodium hydroxide was pulverized by grinding NaOH pellets in a domestic coffee grinder.

Preparation of 6-*O*-(Triphenylmethyl)cellulose ("Tritylcellulose")

The preparative method followed that previously described[17,21]: The completely deacetylated cellulose (32.5 g) was added to 300 mL of dry pyridine, which was then heated for 3 hours at 80°C and filtered off, in order to remove water. This procedure was repeated three times. The cellulose was added to 500 mL of anhydrous pyridine and 142.0 g of triphenylmethyl chloride (2.5 moles per mol of hydroxyl group) in a 1000 mL three-necked round-bottom flask equipped with a condenser, stirrer and dring tube. The mixture was heated for 26 hours at 95°C, then cooled to room temparature and poured into methanol. The precipitate was isolated and washed in methanol for 12 hours. After a second washing, the precipitate was filtered off and dried at 100°C under vacuum. The yield of the product was 95% and the degree of substitution with trityl groups was 1.07 by ^{1}H-NMR measurements.

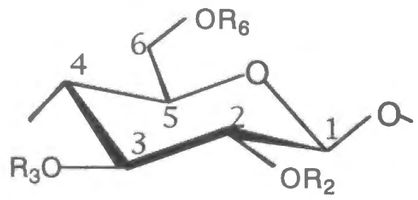
Preparation of Tri-*O*-methyl and Tri-*O*-ethylcelluloses

The preparative route to these derivatives has been described in Chapter 1[15].

Preparation of Partially Alkylated Celluloses at the C-2 and C-3 Positions (Figure 2-1. mixture of S_0, S_2, S_3 and S_{23})

Tritylcellulose (1.0 g) completely dissolved in 60 mL of DMSO on stirring for 1 hour at 60°C. The solution was cooled to room temperature, and 2.0 g of powdered NaOH was dispersed in it. Following 1 hour's stirring under a nitrogen atmosphere, the

alkyl iodide (3.1 mL of methyl iodide or 4.0 mL of ethyl iodide) was added dropwise to the solution. The amount of powdered NaOH and alkylating agents were adjusted according to 10 moles per mole of hydroxyl groups in tritylcellulose. The mixture was stirred under a nitrogen atmosphere for 1 hour, kept at 40°C for 1 hour, at 50°C for 1 hour and at 60°C for 2 hours. The reaction mixture was cooled to room temperature and poured into 95 % methanol. The precipitate was dissolved in chloroform, the chloroform layer was extracted with water three times, and then evaporated under reduced pressure at 60°C to a syrup. The products (MTC1 for methylation and ETC1 for ethylation) were precipitated from the syrup by the addition of methanol, filtered off, and dried under vacuum at 65°C. The yield of the product was 90 %.



	R_2	R_3	R_6	
(1)	H	H	H	$=S_0$
(2)	Alkyl	H	H	$=S_2$
(3)	H	Alkyl	H	$=S_3$
(4)	Alkyl	Alkyl	H	$=S_{23}$
(5)	Alkyl	Alkyl	Alkyl	$=S_{236}$

Figure 2-1. The structures of five of the possible types of glucose residues found in cellulose ethers.

As the alkylation procedure did not result in complete substitution at both the C-2 and C-3 positions, it was repeated three times. For the second alkylation, the procedure was as follows: One gram of MTC1 or ETC1 was added to 60 mL of DMSO, and following stirring for 1 hour at 60°C the turbid solution was cooled to room temperature. The same amount of powdered NaOH used to prepare MTC1 and ETC1

was added to the solution. After the mixture was stirred for 1 hour, 3.1 mL of methyl iodide or 4.0 mL of ethyl iodide was added dropwise. Following 30 minutes stirring under a nitrogen atmosphere at room temperature, the temperature was raised to 70°C and kept there for 3 hours. The reaction mixture was then cooled to room temperature, and the product was precipitated and purified according to the procedure given above.

After a total of four alkylations the hydroxyl groups at C-2 and C-3 were still only partially substituted. Samples of methylated tritylcellulose (MTC1 - MTC4) and ethylated tritylcellulose (ETC1 - ETC4) were isolated after each of the alkylation step, and each of these samples was detritylated by treating 4.5 g of the polymer in 150 mL of dichloromethane with hydrogen chloride gas for 4 minutes[18]. The detritylated mixture was poured into 500 mL of acetone. The products (MC1 - MC4 for four methylated celluloses and EC1 - EC4 for 4 ethylated celluloses) were isolated by centrifugation, washed with acetone, filtered and dried under vacuum at 65°C. The yields of the detritylated products were approximately 85 %.

Preparation of 2,3-Di-*O*-alkylcellulose (Figure 2-1. S_{23})

The method[15] for the preparation of tri-O-alkylcelluloses was applied to the preparation of 2,3-di-*O*-alkylcelluloses. Thus, 2.5 g of powdered NaOH was dispersed in a solution of tritylcellulose(1.0 g) in DMSO (60 mL) containing 1 mL of water. Following 1 hour's stirring under a nitrogen atmosphere, two thirds of the total amount of alkyl iodide (2.2 mL of methyl iodide and 2.8 mL of ethyl iodide) was added dropwise to the solution. At 2,3 and 4 hours after first addition of alkyl iodide, one third of the remaining alkyl iodide (0.3 mL of methyl iodide or 0.4 mL of ethyl iodide) was added dropwise. Following the last addition of alkyl iodide to the solution, still under a nitrogen atmosphere, the temperature was raised to 70°C and kept there for 20 hours. The mixture was cooled to room temperature and the product was isolated and purified as described above. The yield of the product was 90 %. Following alkylaton the products were detritylated and samples (2,3-di-*O*-MC for 2,3-di-*O*-

methylcellulose and 2,3-di-*O*-EC for 2,3-di-*O*-ethylcellulose, respectively) were isolated by the same method as descibed in the previous section.

Preparation of 2,3-Di-*O*-alkyl-6-*O*-partially Alkylated Cellulose (Figure 2-1. mixture of S_{23} and S_{236})

2,3-Di-*O*-alkylcelluloses were treated in DMSO by the same method as described for the first alkylation in the methylation or the second alkylation in the ethylation of tritylcelluloses. In this reaction, the hydroxyl groups at the C-6 position were expected to be alkylated. After the alkylation, the reaction mixture was extracted with 100 mL of each of water and methylene chloride. The methylene chloride layer was separated, washed with water and evaporated under reduced pressure at 40°C to yield a syrup. The product was precipitated from the syrup by the addition of aqueous 50 % acetone. The filtered product was dried first in the air then under vacuum at 65°C. The alkylation step was repeated three times. A sample (2,3-di-*O*-MC1 - 2,3-di-*O*-MC4 for methylation and 2,3-di-*O*-EC1 - 2,3-di-*O*-EC4 for ethylation) was isolated after each alkylation step.

Analyses

The distribution of methyl and ethyl groups in the MC and EC samples was determined by acid hydrolysis and analysis of the components as partially alkylated alditol acetates by gas-liquid chromatography (GLC) [3,22]. The sample (50 mg) was dissolved in 3 mL of 72% sulfuric acid, kept at room temperature for 1 hour, then diluted into 3 % sulfuric acid and heated at 120°C for 1 hour. The hydrolyzate was neutralized to pH 5.5 with barium hydroxide and the precipitated barium sulfate was removed by centrifugation. The partially alkylated glucoses thus obtained were reduced with $NaBH_4$ and then acetylated. The alditol acetate were were dissolved in $CHCl_3$ and analyzed with a Hewlett-Packard Model 5890A gas-liquid chromatograph equipped with a Hewlett-Packard Model 3392A integrator, a flame-ionization detector, and a J. and W. Scientific DB-1 fused-silica capillary column (0.25 mm x 30 m; silicone film thickness 0.25 μm). The average area percent of triplicated analyses was

adopted as the area percent of each peak. The temperature was increased from 190°C to 210°C at 1°C / min..

IR spectra were obtained with a Mattson Cygnus FT-IR spectrophotometer using photoacoustic detection. ^{13}C-NMR spectra were obtained with a Varian XL 200 spectrometer operating at 50.4 MHz, on samples dissolved in deuterated chloroform ($CDCl_3$) and pyridine-d_5 (C_5D_5N). Chemical shifts were measured from the solvent signal (δc 77.01 for $CDCl_3$) and from internal tetramethylsilane, respectively.

Size exclusion chromatograms in chloroform and tetrahydrofuran were obtained with a refractive index detector and 10^5, 10^4, 10^3, 500 and 100 Å μ-Styragel colums in series. The column was calibrated with a series of polystyrene standards.

RESULTS AND DISCUSSION

FT-IR and ^{13}C-NMR Analyses of the Products

Cellulose samples partially alkylated at the C-2 and C-3 positions were prepared from alkylated tritylcellulose. Repeating the alkylation in dry DMSO solutions increased the degree of substitution of the OH groups. However, the IR spectra of Figures 2-2 and 2-3 show that OH streching bands around 3400 cm^{-1} decreased with repeated alkylation, but did not disappear. In other words, neither 2,3-di-*O*-MTC nor 2,3-di-*O*-ETC could be prepared by this procedure. On the other hand, after alkylation of tritylcellulose in DMSO solution containing a small amount of water, the hydroxyl stretching band almost disappeared for the methylated sample (Figure 2-2, trace 6), and was absent from the spectrum of the ethylated sample (Figure 2-3, trace 5), suggesting that 2,3-di-*O*-alkyl-6-*O*-tritylcelluloses were prepared in one step.

Significant depolymerization of the product did not occur during alkylation. The size-exclusion chromatograms showed no significant change with number of alkylation steps (Figure 2-4), so serious chain degradation did not result at this stage. Following alkylation, the samples were detritylated with HCl gas, all under identical conditions.

All the *O*-alkylcellulose samples thus prepared should have had almost the same degree of polymerization even if some acid-catalyzed degradation of the cellulose did occur.

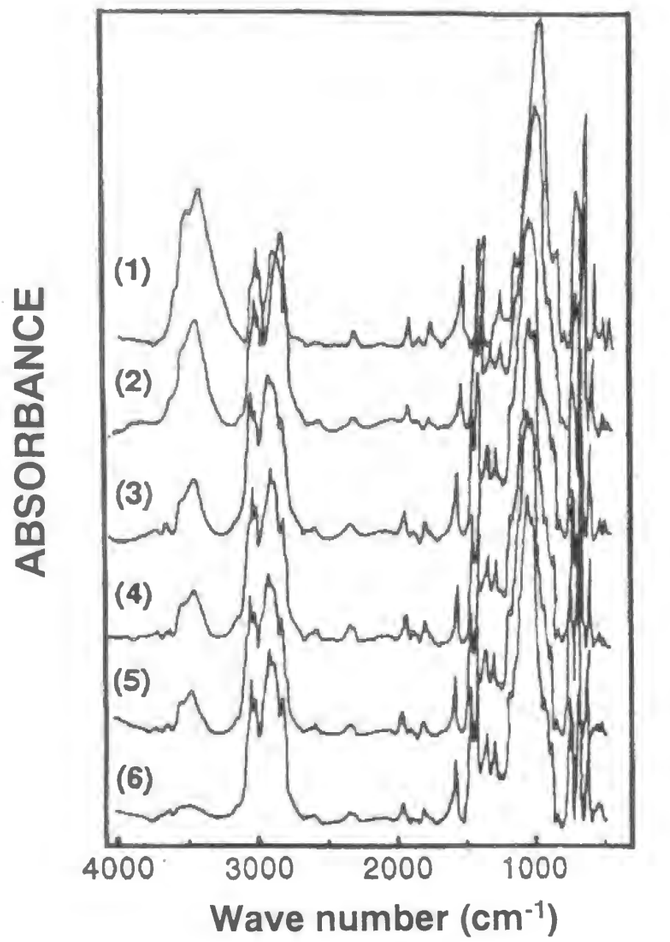


Figure 2- 2. The changes in the IR spectra of tritylcellulose with repeated methylation.
(1): Tritylcellulose, (2) - (5): Methylated tritylcelluloses with increasing methylation, (6): 2,3-di-*O*-methyl-6-*O*-tritylcellulose.

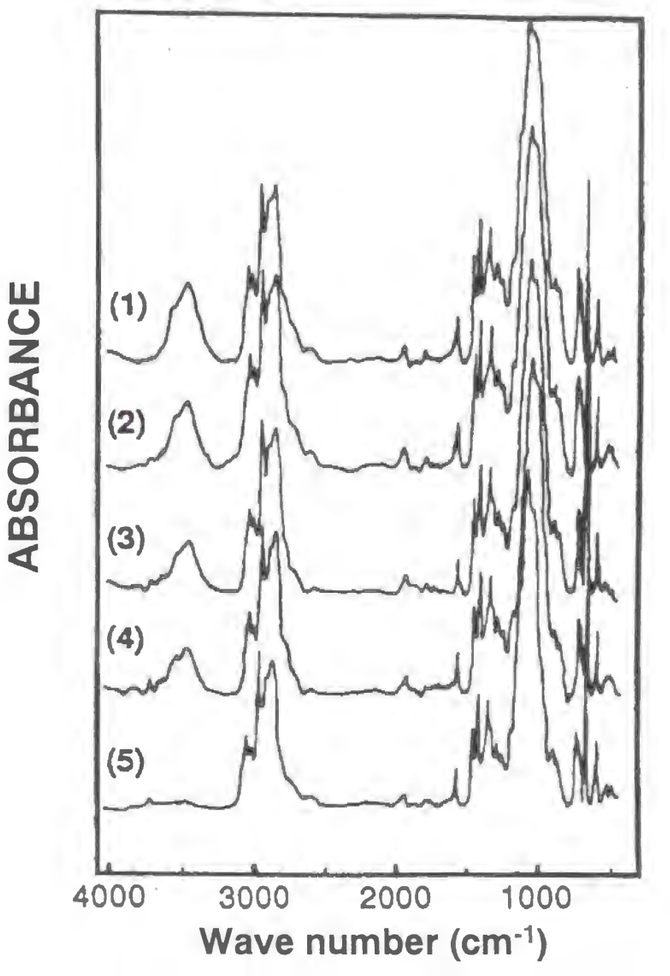


Figure 2-3. The changes in the IR spectra of tritylcellulose with repeated ethylation.
(1) - (4): Ethylated tritylcelluloses with increasing ethylation,
(5): 2,3-di-*O*-ethyl-6-*O*-tritylcellulose.

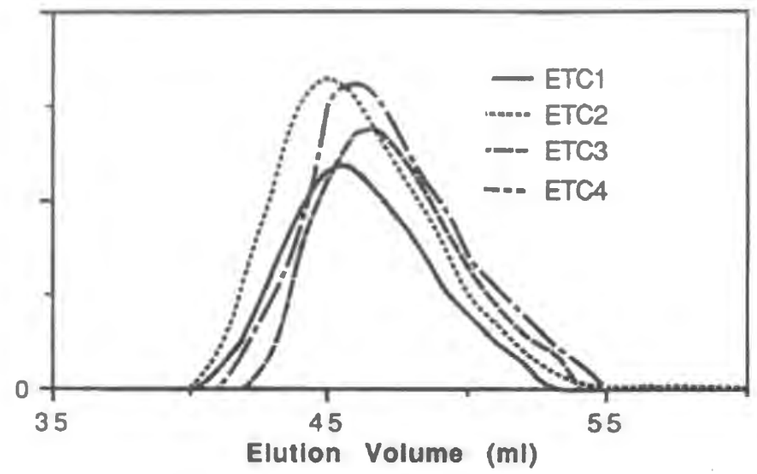


Figure 2-4. Size exclusion chromatograms of tritylcellulose samples after one (ETC1) to four (ETC4) ethylation reactions.

The 50.4 MHz ^{13}C-NMR spectra for both 2,3-di-*O*-ethylcellulose (2,3-di-*O*-EC) and 2,3-di-*O*-ethyl-6-*O*-tritylcellulose (2,3-di-*O*-ETC) are shown in Figures 2-5a and 2-5b, respectively. The signals appearing at 16.5 ppm is assigned to the methyl carbon of the ethyl substituents. The signals for the methylene carbons in the ethyl substituents were located around 67 and 69 ppm in the respective spectra. The signal for C-6 appears at 62 ppm[8]. Carbon atoms substituted by ethoxy groups (C-2 and C-3) gave a broad signal around 84 ppm in 2,3-di-*O*-ETC, and two signals at 84.4 and 83.6 ppm, in 2,3-di-*O*-EC. The signals of carbon atoms bearing ethoxy substituents exibited about 9 ppm of downfield shift relative to the corresponding carbons of cellulose itself[7,8,23,24]. Signals for C-4 and C-5 appear around 77-79 ppm and the signal at 103.8 ppm was assigned to C-1. The aromatic trityl carbon signals appeared at 128.5, 129.5 and 144 ppm (c,d and b) in Figure 2-5b, and the signal at 86.8 ppm can be assigned to the quaternary carbon of the trityl group[25,26]. The solvent signals appeared around 77.0 for

$CDCl_3$ and, 149.9, 135.5 and 123.5 ppm for C_5D_5N. Comparing spectra a and b in Figure 2-5, signals were sharper in a and no trityl signals appeared there. This suggests that the HCl treatment successfully removed the trityl groups in 2,3-di-*O*-ETC to yield 2,3-di-*O*-EC, but some depolymerization may have occurred during the reactions, resulting in sharper ^{13}C signals. The NMR results were supported by the FTIR spectra

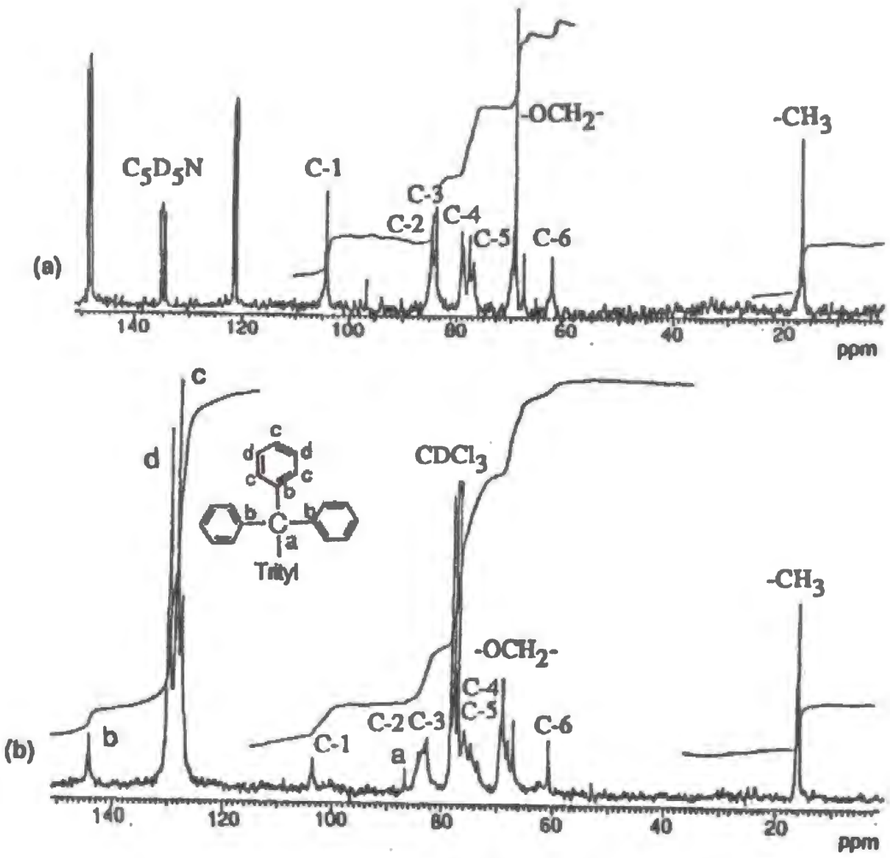


Figure 2-5. ^{13}C-NMR spectra of a; 2,3-di-*O*-ethylcellulose (2,3-di-*O*-EC, Table 2-2) in C_5D_5N at 30°C and b; 2,3-di-*O*-ethyl-6-*O*-tritylcellulose (sample 5, Figure 2-3) in $CDCl_3$ at 30°C.

showing trityl groups (1600, 1490 and 1450 cm^{-1}) before and hydroxyl groups (around 3400 cm^{-1}) after the HCl treatment. The FTIR spectra of the detritylated products had no trityl bands.

Distribution of Substituents in *O*-Methyl and *O*-Ethylcelluloses

Tables 2-1 and 2-2 show the results of GLC analyses of the substituent distribution for the *O*-methylcellulose and *O*-ethylcellulose samples, respectively. The mol fractions X_2, X_3 and X_6 were calculated from the molar fractions of glucitol (S_0) and its mono-, di- and tri-derivatives (S_2 – S_{236}, Figure 1-1) which were present in the hydrolysates of the polymer:

$$X_2 = S_2 + S_{23} + S_{26} + S_{236}$$

$$X_3 = S_3 + S_{23} + S_{36} + S_{236}$$

$$X_6 = S_6 + S_{26} + S_{36} + S_{236}$$

$$DS = X_2 + X_3 + X_6$$

These values (Xn) give the distribution of substituents at each position (C-2, C-3 and C-6) of the ring and hence indicate reactivities of hydroxyl groups as indicated in Chapter 1.

For samples MC1 - MC4 and EC1 - EC4 in Table 2-1 and 2-2, the main glucitol residues were S_0, S_2, S_3 and S_{23}, since trityl substituents were used as protective groups, and hence the OH groups at the C-6 position of the products should be free. According to Hearon and coworkers[17], under moderate conditions trityl chloride reacts 13.8 times faster with the primary OH at the C-6 position as with the secondary OH at the C-2 and C-3 positions. This results in degrees of tritylation of approximately 1.0, with 90% substitution at the primary position. In the present procedure, the unreacted hydroxyl groups in the tritylcellulose were almost completely substituted by the alkylation reactions, as the IR spectra for 2,3 di-*O*-MTC and 2,3-di-*O*-ETC (Figure 2-2, trace 6,

Table 2-1. Distribution of substituents in *O*- methylcellulose samples.

		Molar Ratios[b] (%)								DS in Each Position[c]		
Sample	DS[a]	S_0	S_2	S_3	S_6	S_{23}	S_{36}	S_{26}	S_{236}	X_2	X_3	X_6
MC1	1.29	13.7	31.8	12.5	0	42.0	0	0	0	0.74	0.55	0
MC2	1.48	7.9	26.3	9.6	0	56.2	0	0	0	0.82	0.66	0
MC3	1.57	6.2	21.1	9.7	0	63.0	0	0	0	0.84	0.73	0
MC4	1.56	6.8	20.0	9.8	0	63.4	0	0	0	0.83	0.73	0
2,3-di-O-MC	1.77	2.0	10.6	10.0	0	75.0	0	0	2.4	0.88	0.87	0.02
2,3-di-O-MC1	2.87	0	0	0	0	11.6	1.2	1.0	86.3	0.99	0.99	0.89
2,3-di-O-MC2	2.89	0	0	0	0	8.5	1.0	0.9	89.6	0.99	0.99	0.92
2,3-di-O-MC3	2.96	0	0	0	0	2.9	0	0.9	96.2	1.00	0.99	0.97
2,3-di-O-MC4	2.97	0	0	0	0	2.4	0	0.8	96.7	1.00	0.99	0.98
Tri-O-MC	3.00	0	0	0	0	0	0	0	100.0	1.00	1.00	1.00

[a]Equals $X_2+X_3+X_6$. [b]Determined by GLC. [c]X_n is the mol flaction of glucitol derivatives substituted on the OH of Cn (n = 2,3,and 6). For examples, $X_2 = (S_2+S_{23}+S_{236})/100$

Table 2-2. Distribution of substituents in *O*- ethylcellulose samples.

		Molar Ratios[b] (%)								DS in Each Position[c]		
Sample	DS[a]	S_0	S_2	S_3	S_6	S_{23}	S_{36}	S_{26}	S_{236}	X_2	X_3	X_6
EC1	1.13	22.3	34.0	11.1	0	30.6	0	0	2.0	0.67	0.44	0.02
EC2	1.37	14.5	29.2	7.6	0	45.5	0	0	3.2	0.78	0.56	0.03
EC3	1.65	6.0	20.9	5.9	0	62.9	0	0	4.3	0.88	0.73	0.04
EC4	1.61	7.7	21.6	5.0	0	63.5	0	0	2.1	0.88	0.71	0.02
2,3-di-O-EC	1.93	0	8.8	5.7	0	77.2	0	0	8.3	0.94	0.91	0.08
2,3-di-O-EC1	2.57	0	1.6	0.5	0	32.9	1.3	2.5	61.2	0.98	0.96	0.65
2,3-di-O-EC2	2.80	0	0	0	0	16.7	0	2.9	80.4	1.00	0.97	0.83
2,3-di-O-EC3	2.85	0	0	0	0	12.3	0	3.1	84.6	1.00	0.97	0.88
2,3-di-O-EC4	2.88	0	0	0	0	9.7	0	2.0	88.3	1.00	0.98	0.90
Tri-O-EC	3.00	0	0	0	0	0	0	0	100.0	1.00	1.00	1.00

[a,b,c]See Table 2-1.

and Figure 2-3, trace 5, respectively) showed very small hydroxyl absorption bands at 3400 cm^{-1}. (In the case of complete ethylation of tritylcellulose, there was no OH bands around 3400 cm^{-1} and hence complete substitution.) Since the DS with respect to ethyl groups of this 2,3-di-*O*-ETC is 1.93, corresponding to the result for 2,3-di-*O*-EC in Table 2-2, the DS of tritylcellulose should be 1.07. This value agreed with ^{1}H-NMR measurements. The values of the molar fraction of S_2, S_3, S_{23} and S_{236} from 2,3-di-*O*-EC in Table 2-2 should also be equal to the substitution ratios of trityl groups in C-3 plus C-6, C-2 plus C-6, C-6, and nonsubstituted positions, respectively. Thus, it may be inferred on the basis of Eq. 1 that tritylation occurred with 92 % substitution of the primary OH groups on C-6, 9 % substitution of the secondary OH on C-3, 6 % substitution at the C-2 position, and no substitution of 8 % of the units. The order of reactivity is thus C-6 >> C-3 > C-2.

With the alkylation steps yielding MC1-MC3 and EC1-EC3 the values of total DS and molar fraction of S_{23} increased, while S_2 and S_3 decreased. After three alkylation steps the distribution patterns did not change, suggesting that alkylation does not proceed further.

Repeated alkylations in dry DMSO of samples 2,3-di-*O*-MC and 2,3-di-*O*-EC gave as major products the glucitol residues S_{23} and S_{236} (Tables 2-1 and 2-2). The molar fraction of S_{23} decreased instead of increasing after each alkylation step, indicating that the OH groups at the C-6 positions of S_{23} were being substituted to yield S_{236}. When 2,3-di-*O*-MC and 2,3-di-*O*-EC were alkylated in DMSO containing a little water, they gave the trisubstituted ethers Tri-*O*-MC and Tri-*O*-EC.

CONCLUSIONS

As the alkylation level increased from MC1 to 2,3-di-*O*-MC4 and from EC1 to 2,3-di-*O*-EC4, the OH groups at the C-2 and C-3 positions were substituted progressively to give, after detritylation, 2,3-di-*O*-alkylcelluloses having free OH groups at the C-6

position. These free OH groups were then further alkylated to give nearly completely trisubstituted cellulose ethers. Addition of a limited amount of water allowed virtually complete alkylation of unprotected OH groups in a single step. These polymers would appear to be ideal samples for determining a relationship between physicochemical properties and the distribution of substituents within the anhydroglucose units in *O*-alkylcellulose derivatives.

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Chapter 3

Preparation of 6-*O*-Alkylcelluloses

ABSTRACT: Cellulose derivatives regioselectively substituted at the primary hydroxyl groups, 6-*O*-alkylcelluloses, were prepared from tritylcellulose (trityl=triphenylmethyl). In the preferred procedure, the tritylcellulose was first completely allylated in dimethyl sulfoxide (DMSO), and subsequently detritylated with hydrogen chloride to yield 2,3-di-*O*-allylcellulose. This product was isomerized into 2,3-di-*O*-(1-propenyl)cellulose with potassium *tert*-butoxide in DMSO. The polymer thus prepared was then alkylated completely with methyl or ethyl iodide, in DMSO containing a trace of water. The alkylated polymer was finally treated with 0.1M HCl in aqueous 90 % methanol at room temperature to remove the 1-propenyl groups. The products were shown by Fouriertransform infrared spectroscopy (FT-IR), ^{13}C-nuclear magnetic resonance spectroscopy (NMR), and gas-chromatographic analysis (GLC) to be uniformly substituted at the C-6 position.

INTRODUCTION

The distribution of substituents in cellulose derivatives is considered to be one of most influential factors determining their physicochemical properties such as solubility, crystallization, gel formation, liquid crystal formation, and also resistance to enzymatic degradation. In this connection, the synthesis of cellulose ethers with a regioselective substitution is important in clarifying the relationships between the chemical structure of the derivatives and their physicochemical properties. However, methods for selective chemical modification have been available only for the 6-position (C-6) of

the anhydroglucose units, where operations such as tritylation[1-3], introduction of aldehyde groups[4-6], halogenation[7], and others[8-12] have been carried out. Research activity on *O*-alkylcelluloses has been limited by the difficulty of controlling the distribution of substituents in the preparation of cellulose ethers.

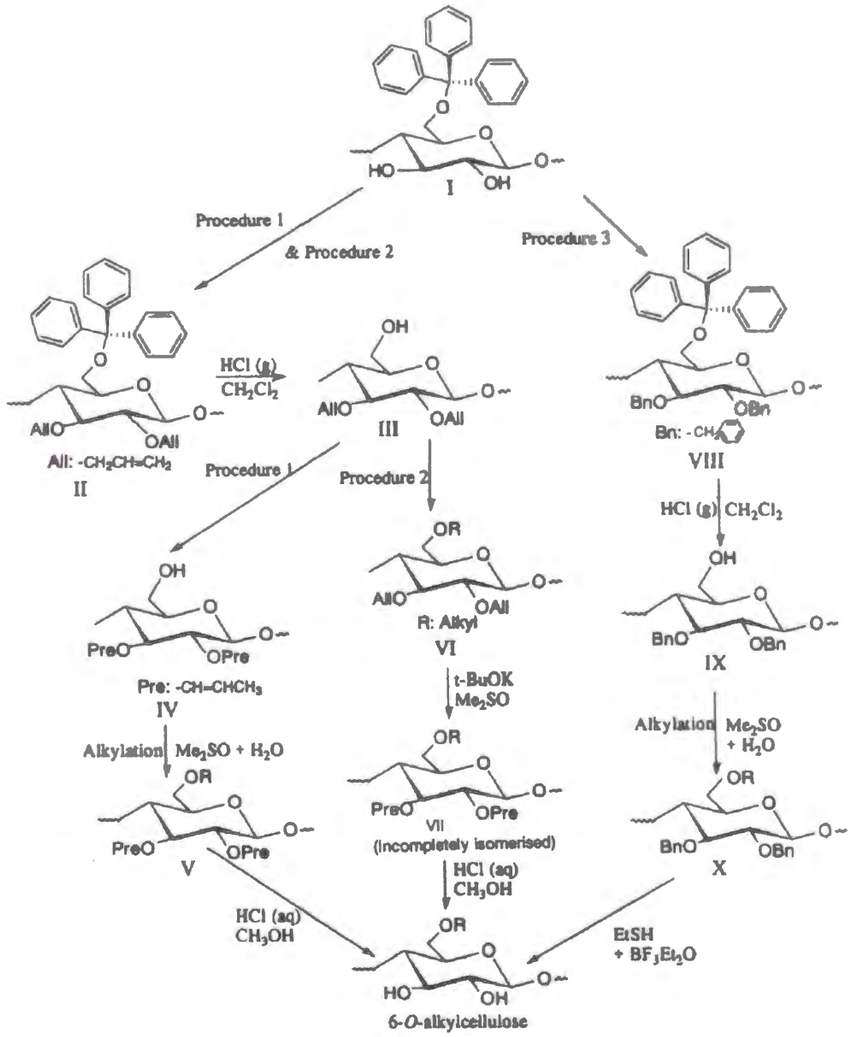
In Chapter 2, the preparation of *O*-methyl and *O*-ethylcelluloses having controlled distribution of substituents has been reported[13]. It was found that the distribution varied systematically in a series of the cellulose ethers prepared by repeated alkylation of 6-*O*-triphenylmethylcellulose ("tritylcellulose"). In the present work, tritylcellulose has been used as the starting material for the preparation of celluloses regioselectively alkylated only at the primary OH groups of the repeating anhydroglucose units (6-*O*-alkylcelluloses). For this purpose tritylcellulose has been converted into 6-*O*-alkyl-2,3-di-*O*-(1-propenyl)cellulose via allylation of the OH groups at C-2 and C-3, followed by detritylation with HCl gas bubbling, then isomerization of the 2,3-di-*O*-allylcellulose into 2,3-di-*O*-(1-propenyl)cellulose, and finally alkylation of the polymer (Scheme 1). In this procedure, the allyl group has been found to be more effective as a protective group than the benzyl group. The products are characterized by GLC of their hydrolyzates as alditol acetates, FT-IR, and ^{13}C-NMR. These measurements have revealed that each of the final polymers has a uniform structure. Because of their uniformity, these 6-*O*-alkylcelluloses as well as 2,3-di-*O*-alkylcelluloses reported in the previous chapter (Chapter 2)[14] are suggested as ideal samples for the study of correlations between physicochemical properties and the distribution of ether substituents.

EXPERIMENTAL

Materials

Commercial cellulose acetate samples were provided by Eastman (acetyl content 39.9 %, ASTM viscosity 27). The samples were completely deacetylated on standing

in aq 15% NH_4OH for 14 days at room temperature. Dimethyl sulfoxide (DMSO) and pyridine were dried over 3A molecular sieves. Reagent-grade solvents and reagents (Aldrich Gold Label) were used without further purification. Powdered NaOH was made by grinding NaOH pellets in a domestic coffee grinder. Water was used after distillation.



Scheme 3-1. Procedures for the preparation of 6-*O*-alkylcelluloses.

Analyses

The distribution of alkyl groups in the alkylcelluloses was determined by acid hydrolysis of samples, conversion of the hydrolyzates into partially alkylated alditol acetates, and analysis by GLC[13,14]. The instrument used was a Hewlett-Packard Model 3392A integrator, a flame-ionization detector, and a J. and W. Scientific DB-1 fused silica capillary column (0.25 mm x 30 m; film thickness 0.25μm). The temperature was increased from 190°C to 210°C at 1°C /min.

IR spectra were obtained with a Nicolet type 7199 FT-IR spectrophotometer. ^{13}C-NMR spectra were obtained with a Varian XL 200 spectrometer at a frequency of 50.4 MHz. Deuterated chloroform and pyridine-d_5 were used as solvents. Chemical shifts were referenced to tetramethylsilane.

Preparation of 6-*O*-Triphenylmethylcellulose ("Tritylcellulose")

The tritylcellulose was prepared according to the method in Chapter 2[13]. The yield of the product was 95 % of the theoretical, and the degree of substitution (DS) by trityl groups was 1.07 by ^{1}H-NMR measurements.

2,3-Di-*O*-allyl-6-*O*-tritylcellulose (II)

The preparative method followed that given in a previous paper[14]: tritylcellulose (3 g) was dissolved completely in 150 mL of DMSO by constant stirring for 2 hours at 60°C. The solution was cooled to room temperature, and 5.94 g of powdered NaOH was dispersed in it. Following 1 hour's stirring under a N_2 atmosphere, allyl chloride (12.1 mL) was added to the solution all at once. The amounts of powdered NaOH and allyl chloride added corresponded to 10 moles per mole of hydroxyl groups in the tritylcellulose. The mixture was stirred under N_2 at 70°C for 4 hours. It was then cooled down to room temperature and precipitated by pouring into aq 95% methanol. The precipitate was dissolved in CH_2Cl_2, the solution was extracted with water three times, and evaporated under diminished pressure at 60°C to a syrup. The product was

precipitated from the syrup by the addition of methanol, filtered off, and dried under vacuum at 65°C. The yield was 95% of the theoretical.

2,3-Di-*O*-allylcellulose (III)

The detritylation procedure followed that given in Chapter 2[14]. 2,3-Di-*O*-allyl-6-*O*-tritylcelluolse (4.5g) (II) was treated with HCl gas in 150 mL of CH_2Cl_2 for 3 minutes at 0°C. The mixture was then poured into acetone (500 mL). The product was isolated by centrifugation, washed with acetone, and dried under vacuum at 65°C. Yields of the detritylated products were ~85%. To test the stability of the double bonds of allyl groups during the treatment, a completely allylated *O*-methylcellulose[14] (DS. by methyl 1.6 and by allyl 1.4) was subjected to HCl gas at both -30 and 0°C and the treated products were characterized by elemental analyses.

2,3-Di-*O*-(1-propenyl)cellulose (IV)

2,3-Di-*O*-allylcellulose (3 g) was completely dissolved in DMSO (150 mL) by stirring for 2 hours at 50°C. With the temperature kept at 50°C, potassium *tert*-butoxide (7.73 g) was added and the solution was stirred for 4 hours under a N_2 atmosphere. The mixture was then poured into acetone. The precipitated product, isolated by centrifugation, was washed with acetone and dried under vacuum at 65°C (yield, 80%).

Alkylation of 2,3-Di-*O*-(1-propenyl)cellulose (IV)

The previously reported method[13,21] for the preparation of completely alkylated cellulose derivatives was applied to 2,3-di-*O*-(1-propenyl)cellulose. The addition of a small amount of water to the DMSO solution was needed. Powdered NaOH (3.1g) was dispersed in a solution of 2,3-di-*O*-(1-propenyl)cellulose (1.5 g) in DMSO (90 mL) containing H_2O (1.5 mL), and after 1hour of stirring under a N_2 atmosphere, two thirds of the total amount of alkyl iodide (e. g., 3.5 mL of ethyl iodide) was added dropwise to the solution. The remaining alkyl iodide was added dropwise in equal portions 2, 3, and 4 hours after the first addition of the reagent. Following the last addition of alkyl

iodide, the solution was kept under a N_2 atmosphere for 20 hours at 70°C. The mixture was then cooled to room temperature. The isolation and purification of the product were accomplished as described above. Yields of the products were~90%.

Removal of 1-Propenyl Groups from 6-*O*-Alkyl-2,3-di-*O*-(1-propenyl)cellulose (V)

6-*O*-Alkyl-2,3-di-*O*-(1-propenyl)cellulose (1 g) was hydrolyzed with 0.1 M HCl in aq. methanol at room temperature[20] for 3 hours. The product was isolated by pouring the mixture into a large amount of cooled water, filtered, and washing the solid several times with distilled water. The final products were dried under vacuum at 65° C and characterized by FT-IR, ^{13}C-NMR, and GLC of the alditol acetates of the hydrolysis products. Yields of the products were 75-80%.

Alkylation of 2,3-Di-*O*-allylcellulose (III)

The procedure employed was the same as that described above for the alkylation of 2,3-di-*O*-(1-propenyl)cellulose.

Isomerization of 6-*O*-Alkyl-2,3-di-*O*-allylcellulose (VI)

O-Alkyldiallylcellulose (1 g) was dispersed in DMSO (60 mL) and then *t*-BuOK (3 g) was added at room temperature with constant stirring. The reaction time was varied in an effort to achieve completion of the isomerization of the allyl groups, as monitored by FT-IR. After the base treatment, the mixture was poured into 1:1 $CHCl_3$-water and the $CHCl_3$ layer was separated. It was washed with distilled water three times, then evaporated under diminished pressure at 60°C to a syrup. The product (VII) was precipitated from the syrup by the addition of methanol, and purified as described for product V.

Removal of 1-Propenyl Groups from Incompletely Isomerized 6-*O*-Alkyl-2,3-di-*O*-(1-propenyl)cellulose (VII)

Treatment and characterization were as described above.

Preparation of 6-*O*-Alkylcellulose via 2,3-Di-*O*-benzyl-6-*O*-tritylcellulose

The benzylation of tritylcellulose followed the procedure reported by Kageyama et al.[27]. Trityl groups were removed as described above to give 2,3-di-*O*-benzylcellulose.

After the polymer was alkylated as detailed above, the product was isolated and a potion (3 g) was debenzylated by treatment with a mixture of ethanethiol and boron trifluoride etherate[22-24]. The solution was evaporated, poured into aqueous $NaHCO_3$, and dialyzed for a week against distilled water. The product was freeze dried, the yield was <10%.

RESULTS AND DISCUSSION

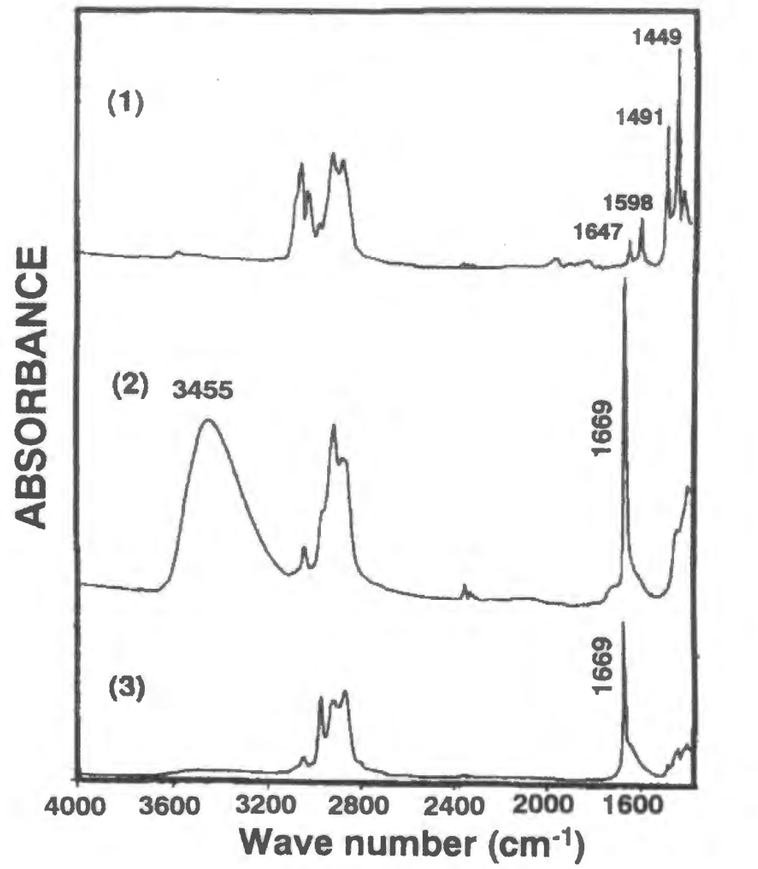


Figure 3-1. IR spectra of (1) 2,3-di-*O*-allyl-6-*O*-tritylcellulose, (2) 2,3-di-*O*-(1-propenyl)cellulose, and (3) 6-*O*-ethyl-2,3-di-*O*-(1-propenyl)cellulose.

To prepare 6-*O*-alkylcelluloses three kinds of procedures (Procedures 1, 2 and 3) were investigated as shown in Scheme 3-1. Procedures 1 and 2 employed allyl groups, and Procedure 3 employed benzyl groups to protect HO-2 and HO-3 of the anhydroglucose units. Each procedure will be discussed in the following section.

6-*O*-Alkylcellulose with Allyl Protective Groups

Both Procedures 1 and 2 needed complete allylation of the HO-2 and HO-3 groups of the tritylcellulose to give 2,3-di-*O*-allylcellulose. The OH groups in the tritylcellulose were completely allylated with a method reported in a previous paper[14]. In the IR spectrum of the product (Figure 3-1, trace 1) no OH absorption band was detected, while C=C absorption bands appeared at 1647 cm^{-1}. The 2,3-di-*O*-allyl-6-*O*-tritylcellulose was then subjected to treatment with HCl to remove trityl groups.

Table3-1. Elemental analyses of allylated O-methylcellulose[a] treated with HCl gas under various conditions.

Treatment	C(%)	H(%)	Cl(%)
Untreated	57.8	7.6	0
−30℃,1h	56.6	7.4	0.6
−30℃,2h	55.1	7.4	1.0
−30℃,3h	54.9	7.3	1.1
0℃,3min	57.6	7.7	0
0℃,1h	57.3	7.7	1.0
0℃,3h	55.8	7.4	1.0

[a]DS by methyl 1.6, by allyl 1.4.

The possibility of a side reaction at this step was explored as recorded in Table 3-1, which shows a comparison of elementary analyses of allylated *O*-methylcellulose before and after HCl treatment under various conditions. This indicates that during detritylation there should be no change in the allyl groups with treatment below 0° C (compare untreated, -30°C, 1hour, and 0°C in Table 3-1). After HCl gas was bubbled in at 0°C for 3 minutes, the IR.spectrum of the product (Figure 3-2, trace 2) confirmed the complete removal of the trityl groups[13,15] (loss of bands at 1598, 1491 and 1449 cm^{-1}, respectively) and the stability of the double bonds in the allyl groups (1647 cm^{-1}).

The allyl ether as a protective group in carbohydrate chemistry has been well-investigated[16-18], because it is conveniently removed by isomerization[19,20] to the *cis*-1-propenyl group and subsequent acid hydrolysis. In this synthesis, this isomerization step was crucial and the following two procedures were employed:

i) Procedure 1; 6-*O*-alkylcelluloses from 2,3-di-*O*-allylcellulose by isomerization prior to alkylation; ii) Procedure 2; 6-*O*-alkylcelluloses from 2,3-di-*O*-allylcellulose by the inverse reaction sequence.

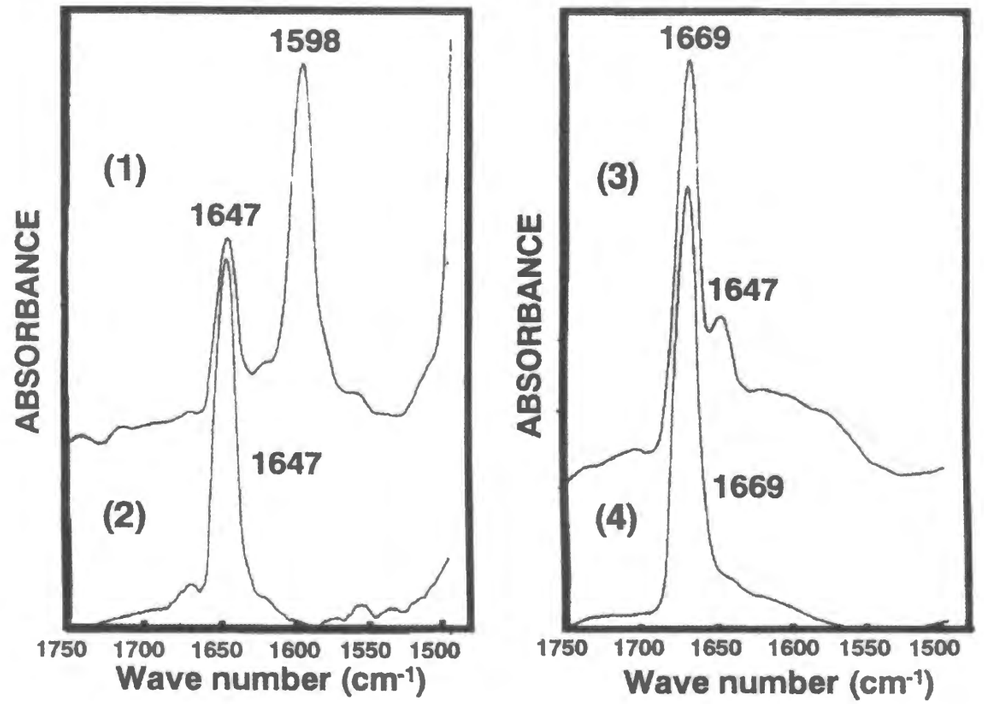


Figure 3-2. Change in the IR spectrum during the isomerization of 2,3-di-*O*-allylcelluloses (C=C region). (1) 2,3-di-*O*-allyl-6-*O*-tritylcellulose, (2) 2,3-di-*O*-allylcellulose, (3) 2,3-di-*O*-allylcellulose after 2 hours' treatment, and (4) 2,3-di-*O*-(1-propenyl)cellulose.

Procedure 1; III-IV-V- 6-*O*-Alkylcelluloses

2,3-Di-*O*-allylcellulose was dissolved completely in DMSO, following which the prototropic rearrangement of the allyl ether groups to their propenyl analogs was effected by employing potassium *tert*-butoxide (*t*-BuOK) as catalyst in a homogeneous solution. IR spectra (Figure 3-2) were used to monitor the process of the isomerization. As shown in traces 2, 3 and 4 of Figure 3-2, the absorption band at 1647 cm^{-1} due to allyl double bonds ($-OCH_2CH=CH_2$) decreased as a function of the reaction time, being replaced by the new band at 1669 cm^{-1} due to 1-propenyl double bonds ($-O-CH=CH-CH_3$). The change in the NMR spectra of the samples during the reaction corroborated these findings. The signals for the two carbons involved in the allyl double bond, which are located around δ136 ($-\underline{C}H=CH_2$) ppm and δ116 ($-CH=\underline{C}H_2$) ppm[14], decreased with the increase of 1-propenyl carbon signals around δ99.5(-O-$\underline{C}$H=CH-) ppm and δ147.5(-O-CH=$\underline{C}$H-) ppm, respectively. The signal of C-1 in the anhydroglucose ring did not shift after the isomerization, suggesting that there was no cleavage of the 1-propenyl ether functions at the neighbouring C-2 position.

Hydroxyl groups at position C-6 in 2,3-di-*O*-(1-propenyl)cellulose were completely alkylated with powdered NaOH and alkyl iodide in DMSO containing a trace amount of distilled water[13,21]. As shown in trace 3 of Figure 3-1, the OH bands around 3455 cm^{-1} disappeared completely, whereas double bonds due to the 1-propenyl groups remained intact (1669 cm^{-1}).

As shown in the IR spectra of 6-*O*-methyl- and 6-*O*-ethyl-celluloses in Figure 3-3, the band at 1669 cm^{-1} almost disappeared after hydrolysis with aqueous HCl (although a trace still remained), and a band due to OH groups appeared around 3465 cm^{-1}. This indicates that the 1-propenyl groups were removed to yield 6-*O*-alkylcelluloses. The hydroxyl bands of both 6-*O*-methyl and 6-*O*-ethylcellulose were located around 3465 cm^{-1}, and 6-*O*-propylcellulose also had an OH absorption band close to the same

position. That is, the stretching vibration bands of the OH groups at the C-2 and C-3 position of cellulose appear to be relatively independent of the length of the alkyl side chain attached to O-6.[22]

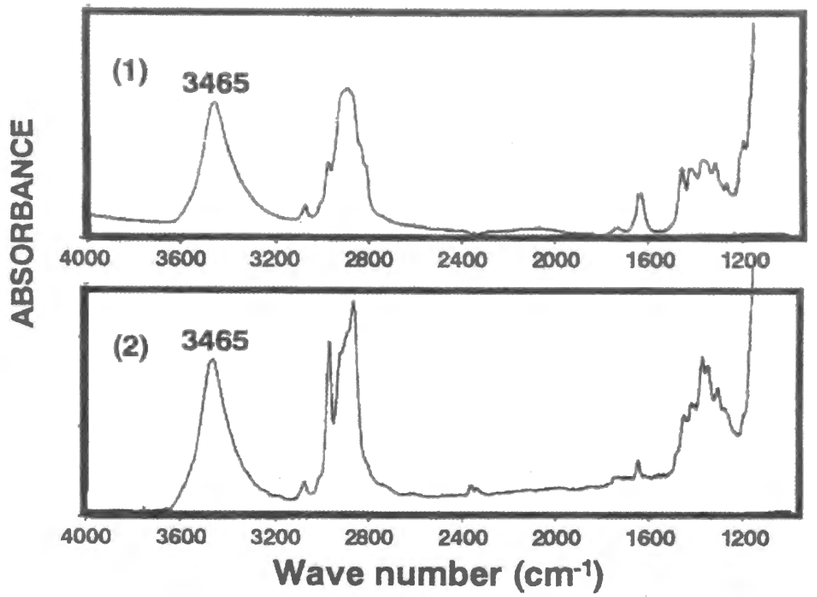


Figure 3-3. IR spectra of (1) 6-*O*-methylcellulose and (2) 6-*O*-ethylcellulose.

Procedure 2; III- VI -VII- 6-*O*-Alkylcelluloses

2,3-Di-*O*-allylcelluose was completely alkylated by the same method used in the previous section. However, the isomerization of allyl groups in the resulting 6-*O*-alkyl-2,3-di-*O*-allylcellulose did not occur completely even when either the reaction temperature or the reaction time was increased. The IR spectra of the products showed patterns similar to those of trace 3 in Figure 3-2. The partial failure of the rearrangement may be due to the insolubility of 6-*O*-alkyl-2,3-di-*O*-allylcellulose in DMSO. In other words, a homogeneous reaction in each step of the procedure for these preparations is preferred.

Procedure 3; Preparation of 6-*O*-Alkylcelluloses via 2,3-Di-O-benzyl-6-*O*-Tritylcellulose

6-*O*-Alkyl-2,3-di-*O*-benzylcellulose was obtained in high yields. However, in the final step, which was the removal of the benzyl groups with ethanthiol and boron trifluoride etherate[23-25], the yield was much less than 10 %. Some depolymerization may have occurred. An alternative method of debenzylation would involve the use of hydrogenation catalysts such as palladium-carbon[26,27], but these might be unsuitable, because of difficulty in the removal of the catalyst from the mixture after the reactions. As a result, it was proved that allyl is superior to benzyl as a protective group in the preparation of 6-*O*-alkylcelluloses.

Uniformity of the Unit Structure in 6-*O*-Alkylcelluloses

To confirm the uniformity of the unit structure of 6-*O*-alkylcelluloses, 6-*O*-ethylcellulose was selected for hydrolysis, and the distribution of its ethyl substituents was determined by the analysis of alditol acetates of the hydrolyzate. Figure 3-4 shows the comparison of GLC pattern of 6-*O*-ethylcellulose and commercially available *O*-ethylcellulose (DS 2.3).

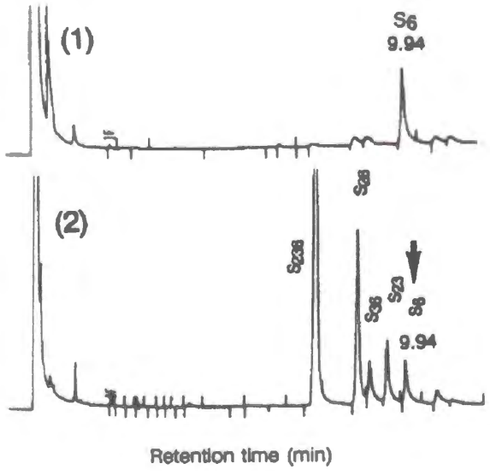
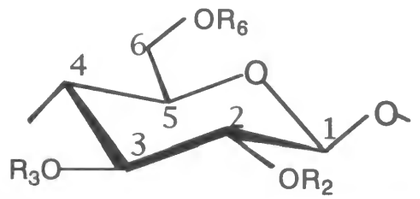


Figure 3-4. GLC patterns of alditol acetates from (1) 6-*O*-ethylcellulose and (2) commercial *O*-ethylcellulose (DS 2.3).
For the meaning of S_0-S_{236}, see Figure 3-5.

Each peak for commercial *O*-ethylcellulose was assigned to one of the eight possible unit structures S_0-S_{236} in Figure 3-5. 6-*O*-Ethylcellulose gave only one peak, assigned to S_6 in which OH groups only at the C-6 position are substituted. This clearly demonstrated that this 6-*O*-alkylcellulose has a uniform structure.



	R_2	R_3	R_6
S_0	H	H	H
S_2	Alkyl	H	H
S_3	H	Alkyl	H
S_6	H	H	Alkyl
S_{23}	Alkyl	Alkyl	H
S_{36}	H	Alkyl	Alkyl
S_{26}	Alkyl	H	Alkyl
S_{236}	Alkyl	Alkyl	Alkyl

Figure 3-5. Structures of the 8 possible types of glucose residues in *O*-alkylcelluloses.

CONCLUSION

6-*O*-Alkylcelluloses composed uniformly of 6-*O*-alkylglucose residues have been prepared from allylated 6-*O*-tritylcellulose, by rearrangement of the allyl protective groups at the C-2 and C-3 positions of the anhydroglucose units, replacement of the trityl groups by alkyl at the C-6 positions, and subsequent acid hydrolysis. These polymers would appear to be ideal samples for determining a correlation between physicochemical properties and the primary OH groups at the C-6 position in alkylcellulose derivatives.

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Part II

Formation of
Inter- and Intramolecular Hydrogen Bonds

Chapter 4

Hydrogen Bonds in Regioselectively Substituted Cellulose Derivatives

ABSTRACT: Formation of hydrogen bonds in various cellulose derivatives, 2,3-di-*O*- and 6-*O*-substituted cellulose ethers, was characterized by FT-IR and CP/MAS ^{13}C-NMR spectroscopies. The polymers were synthesized by regioselective substitution of hydroxyl groups and had a uniform structure. Since their three hydroxyl groups (OH) were selectively blocked, the cellulose derivatives appeared to form specific inter- and intramolecular hydrogen bonds. The characteristic OH stretching frequencies in the IR spectra and the C-1 chemical shift in the CP/MAS spectra of 6-*O*-substituted cellulose derivatives indicated existence of two almost equivalent intramolecular hydrogen bonds between ether oxygen and OH groups at 3-OH---O5' and O6---HO-2' (Figure 4-3(C)). Influence of the substituents at the C-6 position on the formation was not significant except the triphenylmethyl group. Changing behavior of the hydrogen bonding formation in the methylation process of 6-*O*-triphenylmethylcellulose ("tritylcellulose") was also discussed.

INTRODUCTION

Formation of hydrogen bonds in cellulose is considered as one of the most influential factors on physical properties of cellulose and its derivatives. It has been studied extensively by infrared spectroscopy (IR)[1-5] and known in the following way: Cellulose has two different intramolecular hydrogen bonds, which are between the OH-3 and adjacent ring O-5' and between the OH-6 and OH-2' (Figure 4-3A)[3,6-9].

In the previous three chapters, the author reported synthetic methods for regioselective alkylation of hydroxyl (OH) groups in anhydroglucose units of cellulose molecules; the preparation of 2,3-di-*O*-[10], 6-*O*-[11] and tri-*O*-alkylcellulose[12] derivatives (Fig. 4-1). Since the hydroxyl groups should form controlled inter- and intramolecular hydrogen bonds particularly in selectively methylated celluloses, the cellulose derivatives are thought to be cellulose model compounds to investigate relationships between formation of hydrogen bonds and physical properties of cellulose, as well as the cellulose derivatives. This chapter deals with characterization of formation of hydrogen bonds in the regioselectively substituted cellulose derivatives by FT-IR and CP/MAS ^{13}C-NMR spectra of the film samples.

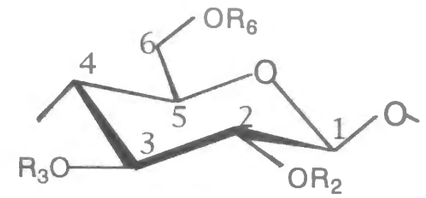
EXPERIMENTAL

Materials

The cellulose samples (Figure 4-1), 2,3-di-*O*-alkyl, 6-*O*-alkyl, tri-*O*- alkylcelluloses and 6-*O*-triphenylmethyl("trityl")cellulose, were prepared, respectively, by the methods reported in Chapters 1-3[10-12]. *O*-Benzyl and *O*-allylcelluloses were prepared in the same manner as the above methods, except that the solvent was water-free in this case[13]. Each polymer had a uniform structure. The average degree of polymerization of each sample was approximately 120 on the basis of the GPC elution curve calibrated with polystyrene standards. Multiple methylation of tritylcellulose was carried out and 2,3-di-*O*-methyl-6-*O*-tritylcellulose was prepared according to the method in Chapter 2 [10]. HPLC-grade *N,N*-dimethyl acetamide (DMAc) (Aldrich Chemical Co., Inc.) was used without further purification.

Preparation of Film Samples

DMAc was used as the common solvent for all samples except tri-*O*-substituted cellulose derivatives. The solution concentration was 0.8 wt%. One gram of each solution was poured into a flat-bottomed tray and heated up to 50°C under a reduced pressure for 3 days. The DMAc solvent was evaporated to yield an as-cast film. It was



	R_2	R_3	R_6
Cellulose	H	H	H
2,3-Di-*O*-	R	R	H
6-*O*-	H	H	R
Tri-*O*-	R	R	R

R: Alkyl, Allyl, Benzyl, and
Trityl (6-*O*-substituted only)

Figure 4-1. The chemical structures of regioselectively substituted cellulose derivatives.
(This figure is the same as Figure 3 in General Introduction.)

further dried for two days under high vacuum at 50° C to completely remove residual DMAc from the film, and stored in a desiccator. A starting cellulose film was prepared by cast of LiCl-DMAc cellulose solution[14], and subsequently washed and dried. For tri-*O*-substituted cellulose derivatives, chloroform was used as solvent and the film was prepared in the same manner. All film samples were prepared as highly non-crystalline ones.

Analyses

Fourier transform infrared (FT-IR) spectra were obtained at room temperature using a Perkin Elmer FT-IR 1720X spectrophotometer. The wave number range scanned was 4000-400 cm^{-1}; 32 scans of 2 cm^{-1} resolution were signal averaged and stored. The intensity with a range of 3100-3700 cm^{-1} was normalized to compare shapes of the

spectra. The films used in this study were sufficiently thin to obey the Beer-Lambert law[15].

The NMR spectrometer used was a JEOL JNM-GX400 operating at 4.7T, which corresponds to resonance frequencies of 100.4 MHz for ^{13}C and 400 MHz for protons. CP/MAS ^{13}C-NMR spectra were obtained at room temperature. Cross polarization times were typically 1-2 ms. The recycle time of the pulse sequence was 10s. The spectra were accumulated ca. 500 times. The chemical shift (29.47 ppm from $(CH_3)_4Si$) of the CH for adamantane crystals was used as an external reference to determine chemical shifts.

RESULTS AND DISCUSSION

FT-IR Characterization

FT-IR spectra of cellulose and regioselectively methylated cellulose films are shown in Figure 4-2 (A). It is found that the shape of the 6-*O*-methylcellulose (6-*O*-MC) is rather symmetric and sharp, compared with that of 2,3-di-*O*-methylcellulose (2,3-di-*O*-MC) which has one OH group per the anhydroglucose unit. Generally increasing the number of OH groups per the unit should show the diversity of OH frequencies in IR spectra and then the absorption band should become broader. However, in the present case the appearance of the OH band for 6-*O*-MC, which has two OH groups per the unit, is contradictory. This phenomenon appeared in other relationships between 6-*O*- and 2,3-di-*O*- substituted cellulose derivatives with the same substituents [Figure 4-2, (B), (C), and (D)]. It is assumed that the phenomenon may be attributed mainly to manner of formation of hydrogen bonds.

Schematic representations of possible inter- and intramolecular hydrogen bonds in the cellobiose unit are shown in Figure 4-3. As described in the Introduction, cellulose may possibly have two types of intramolecular hydrogen bonds and some intermolecular hydrogen bonds, depending on the phase.

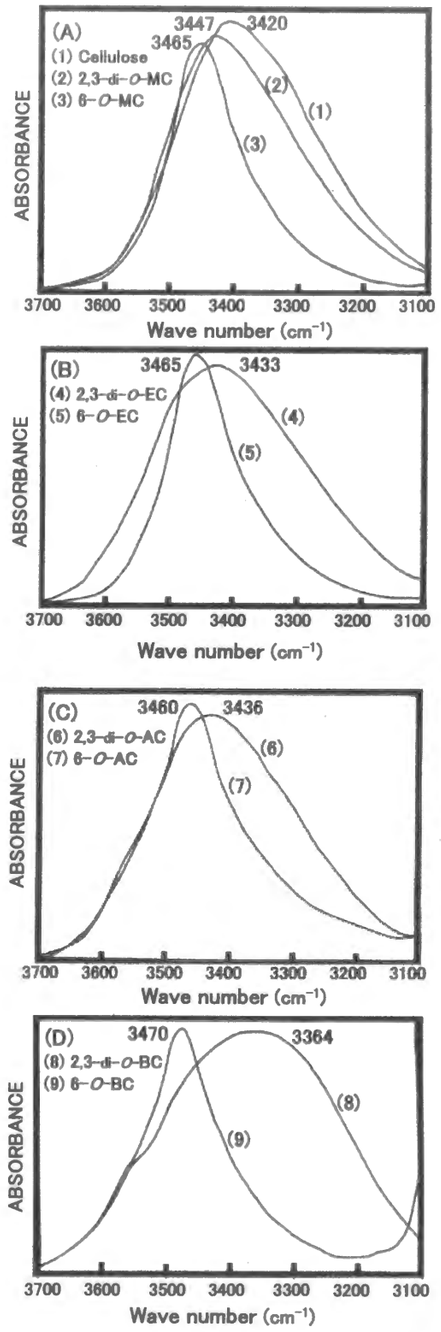
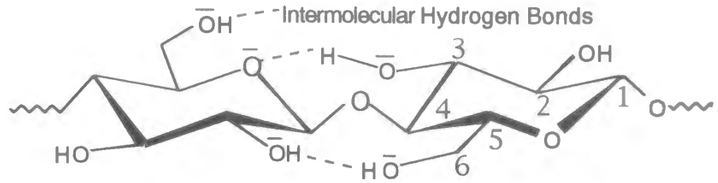


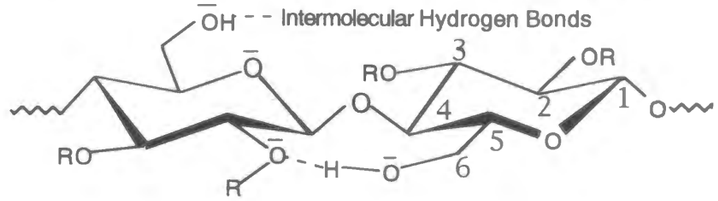
Figure 4-2. IR spectra of film samples of cellulose,2,3-di-*O*- and 6-*O*-substituted cellulose derivatives in the region of OH stretching vibration. MC; *O*-methylcellulose, EC; *O*-ethylcellulose, AC; *O*-allylcellulose and BC; *O*-benzylcellulose.

While the intermolecular hydrogen bonds are not specified, the two intramolecular hydrogen bonds are assumed to be OH-3---O'5 and the OH-6---OH-2' [Figure 4-3 (A)]. In the 2,3-di-*O*-substituted cellulose derivatives, the main reason of the broader OH band due to the diversity of OH frequencies appears to be formation of intermolecular hydrogen bonds associated with the OH-6. In the case of 6-*O*-substituted cellulose, specific intramolecular hydrogen bonding formation, which makes the IR band of the OH region symmetric and sharper, may exist. Hydroxyl and ring ether (C-O-C) groups correspond to the hydrogen bonding donor and acceptor, repectively, which are common in biological structures. When the donor group is a cation-like or the acceptor group is an anion-like, as in $O\text{-}H^{+}\text{---}O$ or $O\text{-}H\text{---}O^{-}$, strong and almost symmetrical hydrogen bonds are also observed[16]. Since in 2,3-di-*O*-substituted cellulose free OH groups at the C-6 position are comparatively flexible, intermolecular hydrogen bonds may be formed favorably. The OH groups may also form intramolecular hydrogen bonds with the ether oxygen at the adjacent C-2 position [Figure 4-3 (B)]. Thus, mixture of the inter- and the intramolecular hydrogen bonds is considered to cause the broadening of the OH band in the IR spectra. On the other hand, two intramolecular hydrogen bonds may form in the 6-*O*-substituted cellulose derivatives as shown in Figure 4-3(C). The two intramolecular hydrogen bonds (O6---HO-2' and 3-OH---O5') are the similar type of the formation, which is between the OH group and the ether or acetal oxygen. Therefore, the sharp and symmetric IR spectra of 6-*O*-substituted cellulose derivatives in Figure 4-2 indicate that the two intramolecular hydrogen bonding structures may give a similar OH absorption band, and intermolecular hydrogen bonding formation which broadens the OH band may be not significant. Furthermore, the strengths of the two hydrogen bonds may be almost equivalent, and hence both of the intramolecular hydrogen bonds between the OH and the ether oxygen appear at almost the same wave number around 3465 cm^{-1} in OH stretching frequencies of the IR bands.

(A) Cellulose



(B) 2,3-di-*O*-Substituted Cellulose Derivatives



(C) 6-*O*-Substituted Cellulose Derivatives

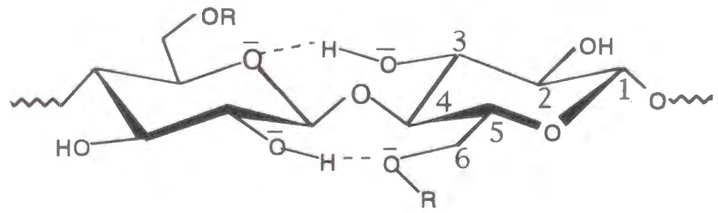


Figure 4-3. Schematic representation of possible hydrogen bonds in cellobiose units of (A) cellulose, (B) 2,3-di-*O*- and (C) 6-*O*-substituted cellulose derivatives.
(This figure has appeared in Figure 4 of General Introduction.)

In another experiment[17], the curve fitting procedure was performed for the regions due to OH stretching vibration in the IR spectra of the non-crystalline films from 2,3-di-*O*-methylcellulose (2,3-di-*O*-MC) and 6-*O*-methylcellulose (6-*O*-MC). The results are shown in Figure 4-4. The OH bands for 2,3-di-*O*-MC are resolved into two Lorentzian bands, sharper (3472 cm^{-1}) and broad (3382 cm^{-1}) ones;

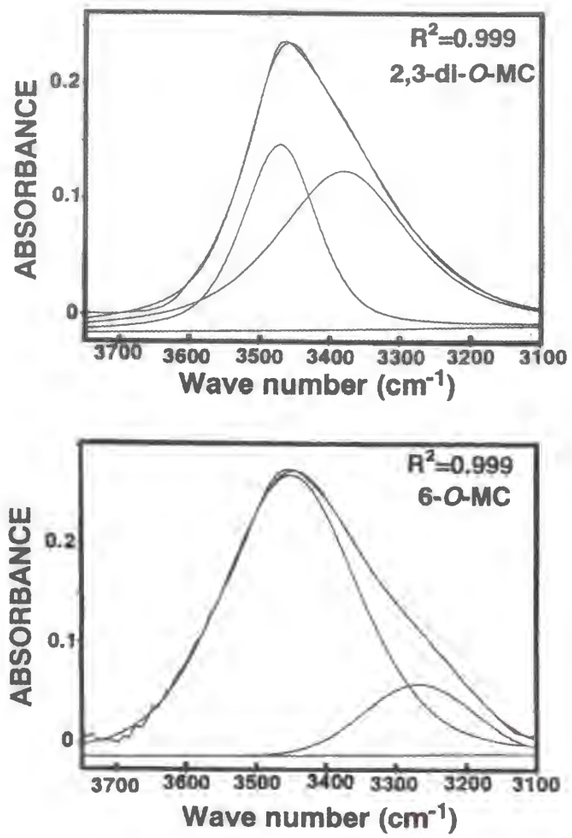


Figure 4-4. Curve fitting for OH stretching regions in 2,3-di-*O*- MC and 6-*O*-MC.

6MC has one major Lorentzian OH absorption band (3460 cm^{-1}) and a small sub-band (3270 cm^{-1}). These bands may correspond to the presence of specific inter- and intramolecular hydrogen bonds involved in 2,3-di-*O*-MC and 6-*O*-MC as mentioned above. Thus, the bands with peak positions at the higher wavenumbers of 3460-3470 cm^{-1} for both 2,3-di-*O*-MC and 6-*O*-MC result in the assignments of the following intramolecular hydrogen bonds, because in general for cellulosic materials the intramolecular hydrogen bonds tend to appear at relatively higher wave numbers

(3410-3460 cm^{-1} in cellulose I[22] and 3460-3480 cm^{-1} in cellulose II[17,26,29]) in the IR spectra. There may be between $\underline{O}CH_3$ at the C-2 position and OH at the C-6 position for 2,3-di-*O*-MC. There may be between OH at the C-3 position and the adjacent ring oxygen and between OH at the C-2 position and $\underline{O}CH_3$ at the C-6 position for 6-*O*-MC.

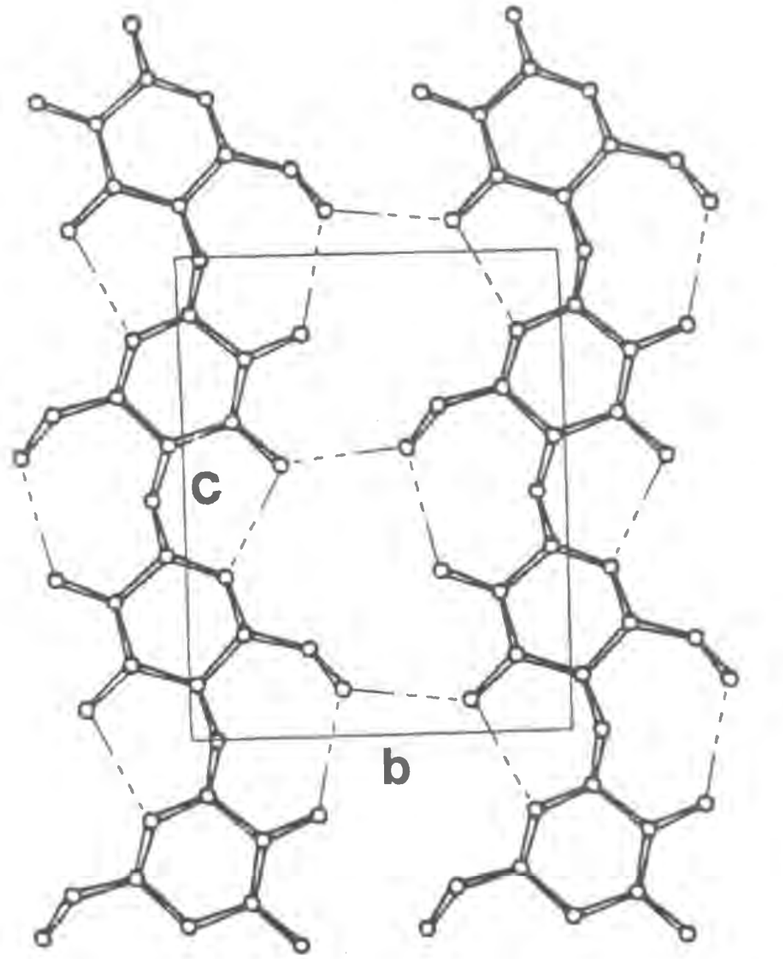


Figure 4-5. Structure of cellulose I: hydrogen bonding network in the sheet parallel to the *bc* plane. This figure is modified on the basis of the Gardner and Blackwell model[18] and the Sarko and Muggli model [19].

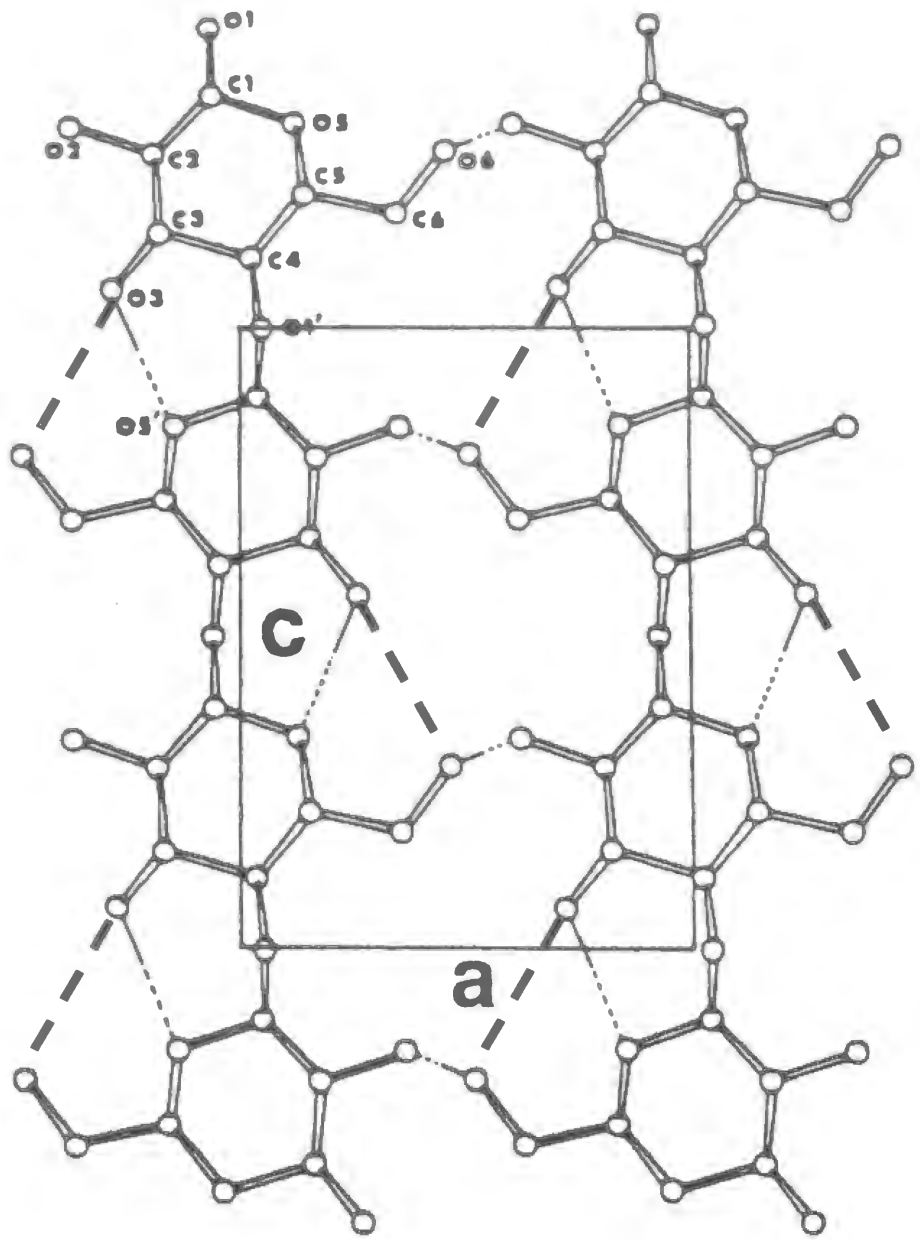


Figure 4-6. A schematic representation of the hydrogen bonds in cellulose II crystalline structure along the c-axis [the a,c-plane (010)], which was proposed by Langan *et al.* in 1999[20]. This figure is modified on the basis of the Kolpack and Blackwell model[19].

Hydrogen bonds are represented by dotted lines.

Table 4-1. IR Assignments for OH regions reported in native cellulose.

Frequency (cm^{-1})	Interpretation (L:by Liang *et.al* 1959)[3] (I: by Ivanova *et.al.*1989)[22] (S: by Sugiyama *et.al.*1991)[23]	Interpretation by Raman (by Wiley *et.al.* 1987)[24]	Calculated wave numbers (by Tashiro *et.al.* 1991)[25]
3230-3310	I: O(6)H--O(3) Inter H-bond		
3231		Cellulose Iα(?) appeared in *Valonia*	
3240	S: Cellulose Iα		
3270	S: Cellulose Iβ		
3305	L: OH Inter H-bond		
3309			OH Inter H-bond
3340-3375	L&I: O(3)H--O(5) intra H-bond		OH Intra H-bond inter--O(3)H--O(5)
3372			
3405	L: OH Inter H-bond		
3410-3460	I: O(2)--O(6) Intra H-bond		OH Intra H-bond O(2)--O(6)--inter
3412			
3429		Cellulose Iβ(?) appeared in *Ramie*	

In Figures 4-5 and 4-6, the views of the cellulose I and II crystalline structures along the c-axis are shown. In cellulose I, the primary OH at the C-6 position is engaged with both inter- (to the OH at the adjacent C-3 position) and intramolecular (to the OH at the C-2 position) hydrogen bonds. The OH group at the C-3 position bears both inter- and intramolecular hydrogen bonds. Only the OH group at the C-2 position is engaged in the single intramolecular hydrogen bond. On the other hand, the primary OH group at the C-6 position in cellulose II has been recently reported[20] to engage in both inter- (to the OH at the adjacent C-2 position) and intramolecular (to the OH at the C-3 position) hydrogen bonds. In this case, the OH group at the C-3 position is supposed to have the dual intramolecular hydrogen bonds, the so-called "three-center type"; 3-OH--O5 (major) and --6-OH (minor)[21]. The OH group at the C-2 position bears only the single intermolecular hydrogen bond. Therefore, OH stretching vibrations in the IR spectra are completely different between cellulose I and II

Table 4-2. IR Assignments for OH regions reported in cellulose II.

Frequency (cm^{-1})	Interpretation (Marchessault *et.al.* 1960)[24]	Calculated wave numbers (Tashiro *et.al.* 1991)[23]
3175	OH stretching	
3305	OH Inter H-bond	
3308		OH Inter H-bond
3309		OH Inter H-bond
3315		OH Intra H-bond
	(Corner chain)	
3350	?	
3374		OH Intra H-bond
3435		(Center chain)
3447	OH stretching	
3486		OH Intra H-bond
		(Corner chain)
3488	Intra H-bond	

crystalline structures, as shown in Tables 4-1 and 4-2 for cellulose I and II, respectively. Here it should be noted that the assignments of OH groups in cellulose II were attempted in previous structural models[27,28] which differ from Figure 4-6. The two resolved Lorentzian bands for 2,3-di-*O*-MC in Figure 4-4 of this study were located in the region with fairly higher wave numbers than the above crystalline structures. This may be due to the noncrystalline phase of the film which has looser packing, and thus causes weaker intermolecular hydrogen bonds. If the primary OH group at the C-6 position, which only has a possibility to form hydrogen bonds in 2,3-di-*O*-MC, bore both inter- and intramolecular hydrogen bonds at the same time, the OH stretching vibration should have appeared as a single Lorentzian band in the IR spectrum. However, the two resolved bands for the OH frequency regions of 2,3-di-*O*-MC indicate that there may not be such a hydrogen bonding OH group. This also indicates that the OH group at the C-6 position in 2,3-di-*O*-MC is not identical to that either in cellulose I or II with regard to the hydrogen bonding situation. The primary OH in 2,3-di-*O*-MC may be engaged in either inter- or intramolecular hydrogen bonds. Judging from the area ratio of the two resolved bands in the film, inter- and intramolecular hydrogen bonds may have the possibility to form approximately at the rate of 55% and 45%, respectively, when the two molar absorption coefficiencies are assumed to be almost identical with each other.

The differences in hydrogen bonds between the two crystals are considered to depend on the type of hydroxymethyl conformation at the C-6 position: the orientation of the C6-O6 bond, *gauche-trans* (*gt*) *or trans-gauche* (*tg*) as shown in Figure 4-7. The conformation for cellulose I has been accepted as *tg*, whereas the type of the conformation for cellulose II has been argued to be the mixture of *gt* and *tg* or *gt* alone.[27-29] Very recently, the structure of cellulose II has been reinvestigated[20,30] and indicated to bear all the hydroxymethyl groups which are in the *gt* situation. When they are *gt*, there is no intramolecular hydrogen bond between OH at the C-2 position and OH at the C-6 position (see Figure 4-7). Thus, in the present study the author's

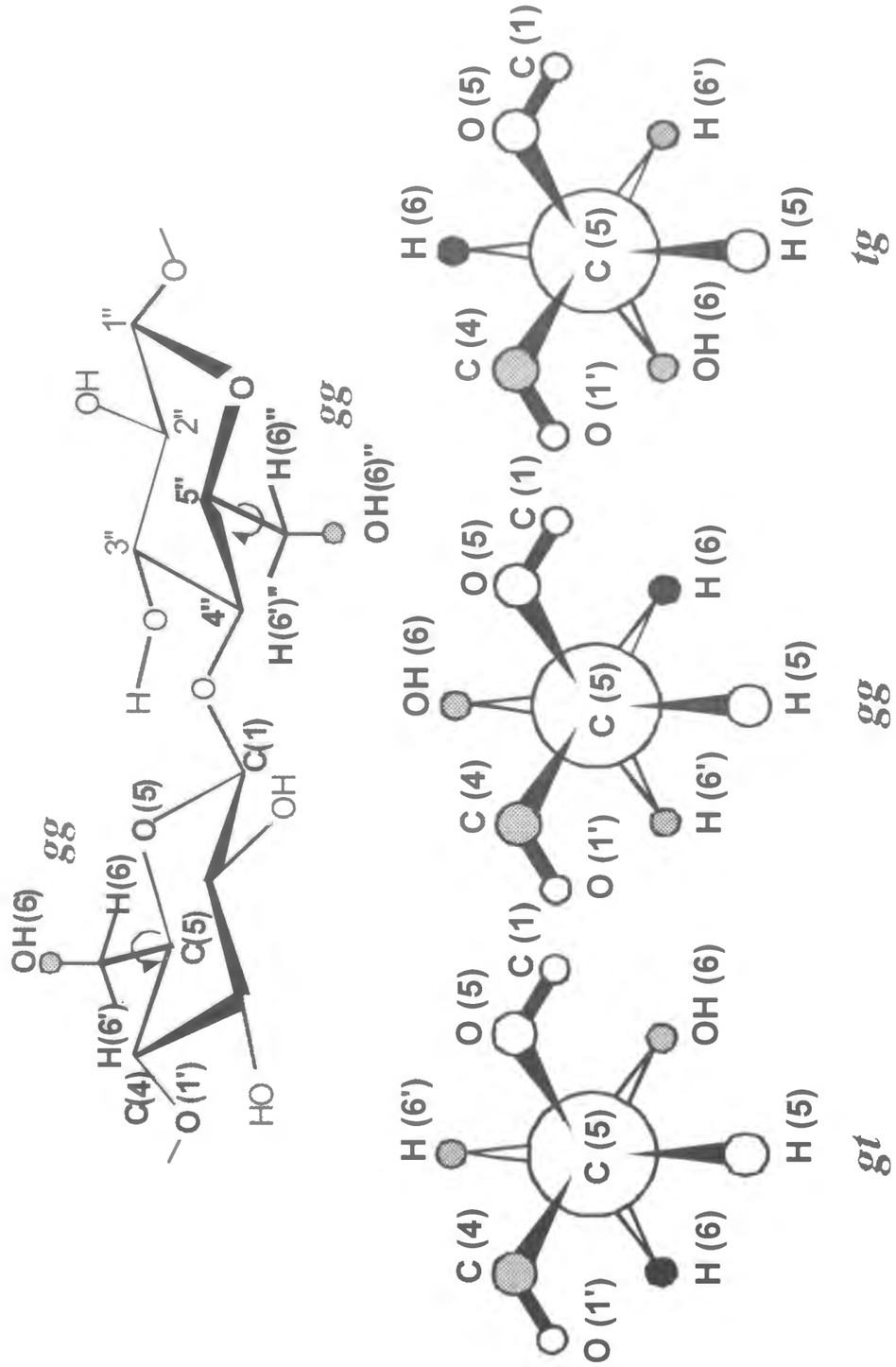


Figure 4-7. The possible conformations of the CH_2OH group at the C-6 position of the anhydroglucose unit.

hypothesis indicates that the regioselectively substituted cellulose derivatives are supposed to have *tg* situation at the rotation of the hydroxymethyl groups at the C-6 position. However, further studies will be required because only the spectroscopic data cannot differentiate completely between the *tg* and *gt* forms, although CP/MAS ^{13}C-NMR[31] and polarlized IR spectra[26] suggested the occurrence. It should be added that if *tg* situation at the C-6 positions in the regioselectively methylated cellulose samples was employed, the phanomena shown in the following chapters (Chapters 5 and 6) could at least be well-explained to be dependent on the intramolecular hydrogen bonds formed due to the conformation.

CP/MAS ^{13}C-NMR Characterization

To confirm the above assumption, the film samples were analyzed by CP/MAS ^{13}C-NMR spectroscopy. Figure 4-5 shows CP/MAS ^{13}C spectra of (a)2,3-di-*O*-MC, (b) 6-*O*-MC and (c) Tri-*O*-MC. The introduction of an *O*-alkyl group promotes strong deshielding of the ^{13}C nucleus of the substituted carbinol group, usually by ca. 9 ppm in solution NMR[17,18]. In the spectra of cellulose ethers, this characteristic should be reflected in the chemical shifts of carbons at C-2, C-3 and C-6 positions bearing alkoxy substituents. Thus, peak assignment was carried out in the CP/MAS ^{13}C-NMR spectra of the film samples using the solution-state ^{13}C-NMR results of Parfondry and Perlin[19]. Unsubstituted C-2, C-3 and C-5, and substituted C-6 carbon signals in 6-*O*-MC overlapped to some extent with each other in the range of 77-70 ppm. Signals of the C-2, C-3 and C-6 carbons shifted to downfield (ca. 10 ppm) by methyl substitution. In the 2,3-di-*O*-MC and Tri-*O*-MC, the C-4 carbon signals, which are assignable in cellulose, shifted upfield by substitution of the adjacent OH groups at the C-3 position and overlapped with the C-5 carbon signal. Signals of C-2 and C-3 carbons in the two MCs overlapped with each other and cannot be identified because of their similar and strong deshielding of ^{13}C nuclei of completely substituted methoxy groups at both positions.

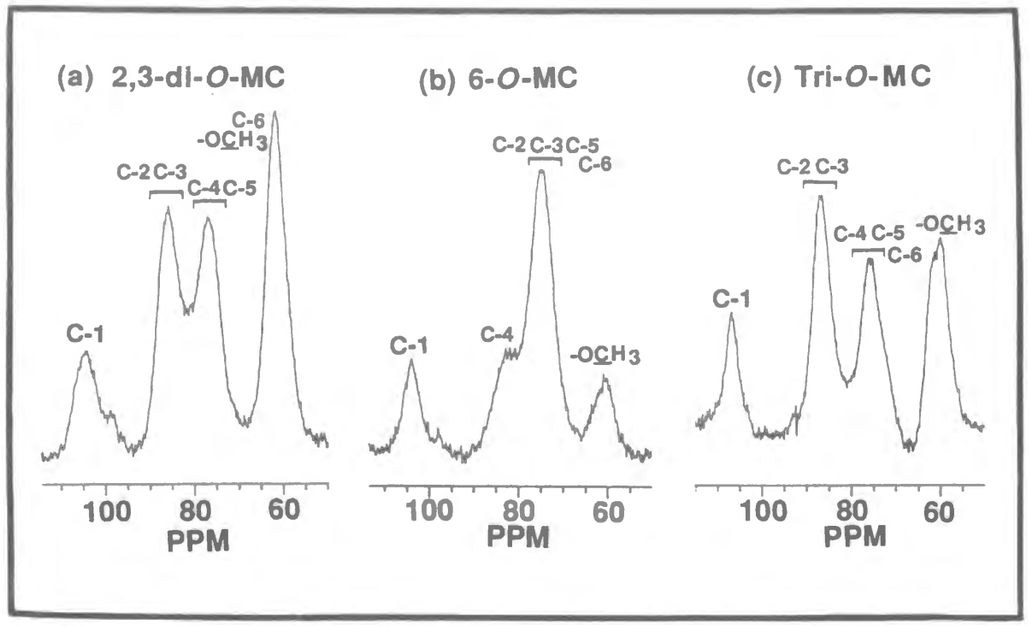


Figure 4-8. CP/MAS ^{13}C-NMR spectra of regioselectively substituted *O*-methylcelluloses (MCs).

Considering a weak deshielding effect of the intramolecular hydrogen bond between the methoxy oxygen at the C-2 position and the OH groups at the adjacent C-6 position as described later, the C-2 carbon may resonate upfield to the C-3 carbon. Only the C-1 signal is easily assignable since it is completely separated from other signals. The presence of an ether substituent at the C-2 position causes an upfield shift of the C-1 resonance relative to that of the glucose residue[34]. Therefore, it is assumed that the C-1 resonances may have an upfield shift similarly by the hydrogen bonding formation at the adjacent C-2 position. Naturally the change by the hydrogen bonding arrangement should be more weakly transmitted to the chemical shift of C-1 carbon than the effect due to the substitution of the OH at the C-2 position. Kamide *et. al*[36] reported that the chemical shift of the C-1 carbon may appear upperfield than 105 ppm in the case of the

Table 4-3. Comparison of chemical shifts at the C-1 positions of anhydroglucose units in CP/MAS 13C-NMR spectra of various cellulose derivatives.

Sample	6-*O*-	2,3-di-*O*-	Tri-*O*-
Methyl Cell.	104.1	104	106.5
Ethyl Cell.	103.8	104	102.2
Propyl Cell.	102.8	105	102.6
Decyl Cell.	104.2	104	LC*
Allyl Cell.	103.1	104	102.5
Benzyl Cell.	103.2	102	102.0
Trityl Cell.	105.5	-	-

*LC: Liquid crystal at room temperature.

intramolecular hydrogen bond between the C-2 and C-3 position [Figure 4-3 (B) and (C)]. They also referred that the C-1 chemical shift may be a lowerfield value than 105 ppm in the case of the free OH at the C-2 position which did not include the hydrogen bond between the C-2 and C-6 position. The latter case was based on the assumption that a seven membered π–σ electron conjugate system is formed in a cellobiose unit [C-4--O--C-1'--O5'--HO-3--C-3--C-4: Consider the case without the intramolecular hydrogen bond between C-6and C-2' in Figure 4-3 (A)]. In Table 4-3, the C-1 chemical shifts of 6-*O*-substituted cellulose derivatives except 6-*O*-tritylcellulose shows smaller values than 105 ppm, suggesting the existence of intramolecular hydrogen bonds between the C-2 and C-6 position. From this result and FT-IR analyses in the previous section, it is indicated that in the 6-*O*-substituted cellulose derivatives except 6-*O*-tritylcellulose, two intramolecular hydrogen bonds (O6---HO-2' and 3-OH---O5') form predominantly and the strengths of the two bonds may be almost equivalent [Figure 4-3 (C)].

The C-1 chemical shifts of 2,3-di-*O*-substituted cellulose derivatives in Table 4-3 also show smaller values than 105 ppm. This indicates the formation of the intramolecular hydrogen bond between O2---HO-6' [Figure 4-3 (B)] in addition to intermolecular hydrogen bonds at the C-6 position. As for tri-*O*-substituted cellulose

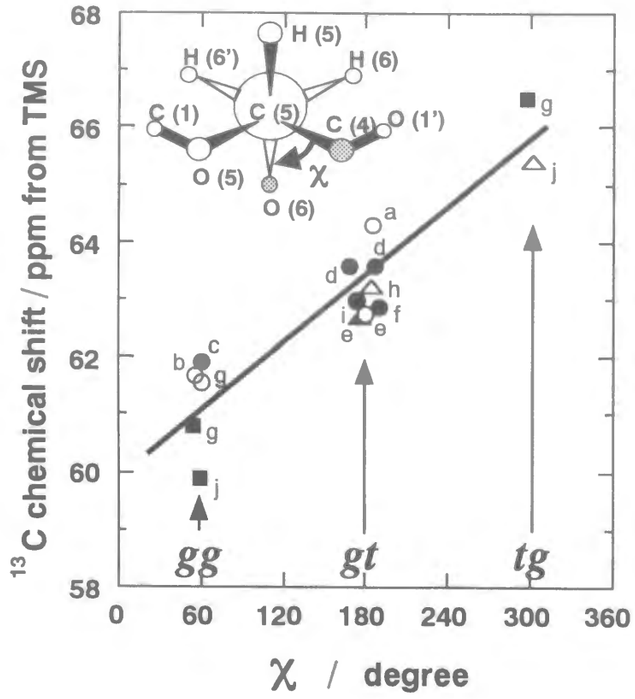


Figure 4-9. ^{13}C chemical shifts of the CH_2OH carbon vs. torsion angles χ around the exo-cyclic C - C bonds.

a: α- D-glucose, b: α- D-glucose • H_2O, c: β- D-glucose,

d: β- D-cellobiose,e: α- D-lactose • H_2O, f: β-lactose, g: sucrose,

h: α- melibiose • H_2O, i: β- methyl cellobioside • CH_3OH,

j: raffinose • $5H_2O$;

○ : α-glucopyranoses, ● : β-glucopyranoses,

△ : α-galactopyranoses, ▲ : β-galactopyranoses,

■ : β-fructofuranoses.

(This figure is a modification of Figure 2 in reference 31 by Horii *et al.*)

ethers, the C-1 signals appeared more upfield than those of 2,3-di-*O*- and 6-*O*-substituted cellulose derivatives. Only tri-*O*-methylcellulose shifted downfield compared with other tri-*O*-substituted cellulose derivatives. To explain these phenomena, the above explanation of chemical shifts with the hydrogen bonding formation effects cannot be simply applied to the C-1 signals of the tri-*O*-substituted derivatives. Because the substitution of both of the C-2 and the adjacent C-6 hydroxyls can cause a mutual repulsion between the two substituents, the interaction occurring among them can be the different type such as hydrophobic linkage and hence the conformation of the main chain should change.

As discussed in the previous section using FT-IR, CP/MAS ^{13}C-NMR may suggest the type of the hydroxymethyl conformations, *gt* or *tg* at the C-6 positions in carbohydrates. Horii *et al.* indicated[31] that the C-6 carbon resonance occurs only as a singlet near 64 ppm in the case of the *gt* conformation whereas a resonance band near 66 ppm will appear when the *tg* conformation is present within the crystalline structures, as shown in Figure 4-9 which was modified from Figure 2 of reference 31. According to them, the chemical shifts fall into three groups of 60-62.6 ppm, 62.5-64.5 ppm, and 65.5-66.5 ppm, which are related to *gauche-gauche (gg), gauche-trans (gt),* and *trans-gauche (tg)* conformations, respectively. In fact, the chamical shift of the C-6 for cellulose II[37-40] indicated the *gt* conformation, which agrees with the recent result from the neutron fiber diffraction analysis[20]. In the regioselectively methylated cellulose ethers shown in Figure 4-8 of this chapter and other experiments[41], the chemical shifts of the C-6 were 61.58 ppm and 61.66 ppm for 2,3-di-*O*-MC and 3-*O*-MC[41], respectively. These results correspond that the conformation of the OH groups at the C-6 position for the two regioselectively methylated cellulose derivatives may be *gg*. However, the indication due to the chemical shifts[31] was derived from mono- and oligosaccharides, which have different hydrogen bonding engagements from the present samples. As mentioned already, even hydrogen bonds for cellulose I and II may be totally different from those for the 2,3-di-*O*-MC and 3-*O*-MC, judging from

the OH stretching frequencies in the IR spectra. Since the relatively large scattering of data within 2 ppm depending on the conformation at the C-6 may be due to other additional effects such as packing[42] and hydrogen bonding[43,44], the chemical shifts of the C-6 for the regioselectively methylated cellulose derivatives, which are expected to have controlled and specific hydrogen bonds, may not agree with the indication. In addition, the conformation of the glucopyranose ring may be somehow changed by the regioselective substitution by methyl groups. Further studies will be required.

Influences of Substituents on the OH Absorption Bands

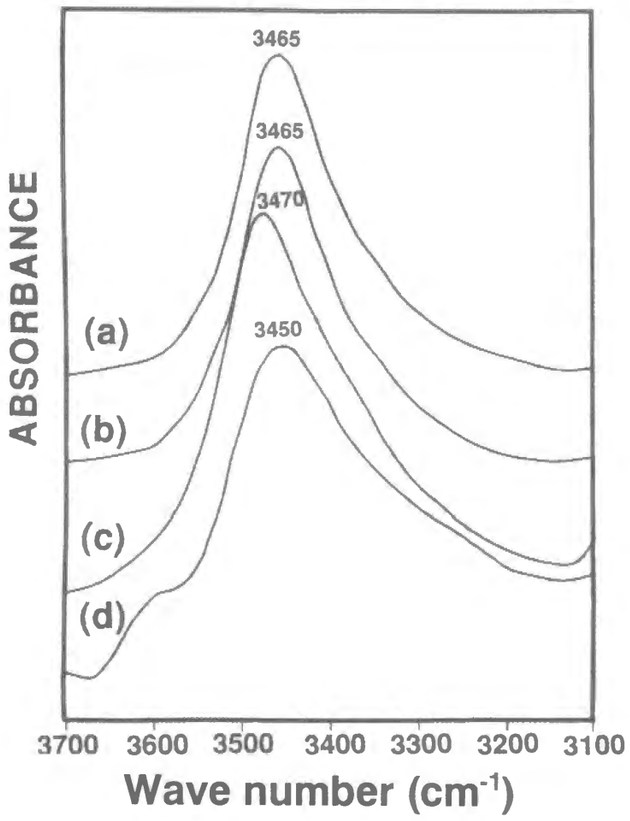


Figure 4-10. IR spectra of 6-*O*-alkylcellulose films in the region of OHstretching frequencies: (a) 6-*O*-methylcellulose, (b) 6-*O*-ethylcellulose, (c) 6-*O*-propylcellulose and (d) 6-*O*-decylcellulose.

Hydroxyl frequencies in IR spectra of 6-*O*-alkylcelluloses with different lengths of alkyl chains are shown in Figure 4-10. The sharp and symmetric shape of the IR spectrum for 6-*O*-MC did not show a significant change with increasing of alkyl chain -length. It suggests that irrespective of the alkyl chain length with the range of one to ten in carbon number, two intramolecular hydrogen bonds expected to be formed in 6-*O*-MC were maintained in 6-*O*-alkylcelluloses. Only in 6-*O*-decylcellulose there appeared a small shoulder around 3600 cm^{-1}. In cases of electron-withdrawing substituents such as allyl and benzyl groups which are easy to form cations [Figure 4-2 (C) and (D)], the hydroxyl frequencies also appeared sharp and symmetric, though there was a slight shoulder at around 3580 cm^{-1}. However, a remarkable shoulder of hydroxyl frequencies appeared at around 3580 cm^{-1} in both an electron-withdrawing and bulky trityl substituent as shown in Figure 4-11. In general hydroxyl frequencies at 3584-3650 cm^{-1} are considered as absorption band of "free" OH groups[36]. Hydroxyl frequencies due to the intermolecular hydrogen bond were reported as 3305, 3350 and 3405 cm^{-1} by Marchessault *et al.*[3,7] The shoulder at 3580 cm^{-1} in the hydroxyl absorption band of the tritylcellulose was found to be due to rather "free" hydroxyl groups. The OH bands should be broad because of the diversity. Thus, an intramolecular hydrogen bond and "free OH" appear to be formed at the C-2 position of the tritylcellulose.

The 6-*O*-tritylcellulose was then multiple-methylated to investigate behavior of OH groups at the C-2 and C-3 positions. Change of distribution of the methyl group as a block of OH groups in a step is monitored in Table 4-4. As OH groups at the C-2 position were rapidly methylated in the first methylation, the degree of substitution (DS) of methyl groups at the C-3 position increased slowly in the step. This difference indicates a quick break of hydrogen bond and methylation of the free hydroxyl groups at the C-2 position, and a slow scission of the intramolecular hydrogen bond between

C-3 and O5 through three methylation steps. Namely, the first apparent change in the IR spectrum of methylated tritylcellulose attributes to the behavior of the hydroxyl group at the C-2 position. In traces of Figure 4-11, as the methylation step proceeded, the intensity of the absorption band at 3484 cm^{-1} decreased gradually and the shoulder at 3580 cm^{-1} got smaller and sharper (In Figure 4-11, the absorbance of the OH bands for the MTCs were normalized on the basis of the internal standard band at 1596 cm^{-1} due to the trityl group which was not affected by the methylation, and the peak heights between 6-*O*-TC (tritylcellulose) and MTC1-3 were not comparable with each other).

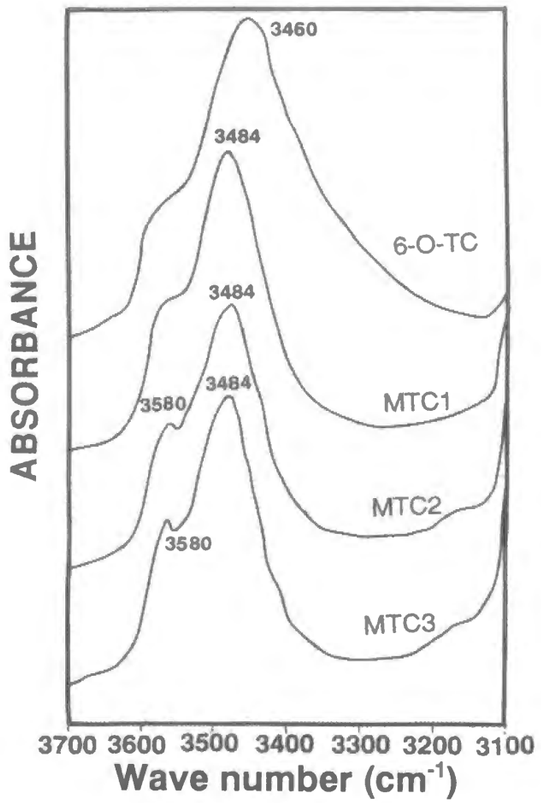


Figure 4-11. Change of OH stretching frequencies in IR spectra of tritylcellulose through the multiple methylation step.
6-*O*-TC : tritylcellulose, MTC1-3: See Table 4-4.

Table 4-4. Degree of substitution (DS) at individual positions in 6-O-tritylcellulose and methylated tritylcellulose derivatives.

Sample	X_2	X_3	X_6
6-*O* -TC	0	0	Trityl
MTC1	0.74	0.55	Trityl
MTC2	0.82	0.66	Trityl
MTC3	0.84	0.73	Trityl

X_n: DS on the OH of C-n (n=2,3, and 6).
MTC: Methylated 6-*O* -tritylcellulose.

Eventually the OH frequencies disappeared and all OH groups were completely methylated to give 2,3-di-*O*-methyl-6-*O*-tritylcellulose. This indicates that once the "free" OH groups were produced from the inter- or intramolecularly hydrogen bonded OH groups at the C-2 position and intramolecular hydrogen bonded OH groups at the C-3 position, then they were easily methylated. Inaddition, the shift of the absorption peak top from 6-*O*-TC to MTCs (from 3460 cm^{-1} to 3484 cm^{-1}) may be due to the variation of the strengths for the intramolecular hydrogen bonds between the OH groups at the C-3 position and the adjacent ring O-5 during the methylation process.

CP/MAS ^{13}C-NMR spectra of the above samples also showed the similar behavior of the OH groups through methylation (Figure 4-12). The broad C-1 signal at 105.5 ppm in the tritylcellulose was located at a relatively downfield when compared with the chemical shifts of other cellulose ethers (Table 4-3), indicating that no intramolecular hydrogen bonds may form between the OH groups at the C-2 position and trityl ether oxygen at the C-6 position. As the methylation proceeded, the signal at the C-1 shifted upfield, resulting in the appearance of three peaks at 105.5, 102.5 and 101.4 ppm in MTC1 [Figure 4-12(2)]. These three C-1chemical shifts may be affected by the neighboring OH groups at the C-2 position, which are "free OH", the intramolecular hydrogen bonded OH, and methyl-blocked OH groups, respectively.

The broad C-1 signal of the tritylcellulose also had a shoulder at around 102.5 ppm due to the intramolecular hydrogen bond. Judging from the C-1 peak shape, the region around 105.5 ppm was main and the vicinity at 102.5 ppm was relatively small. This indicates that the free OH was the main rather than the OH engaged in the intramolecular hydrogen bond at the C-2 position.

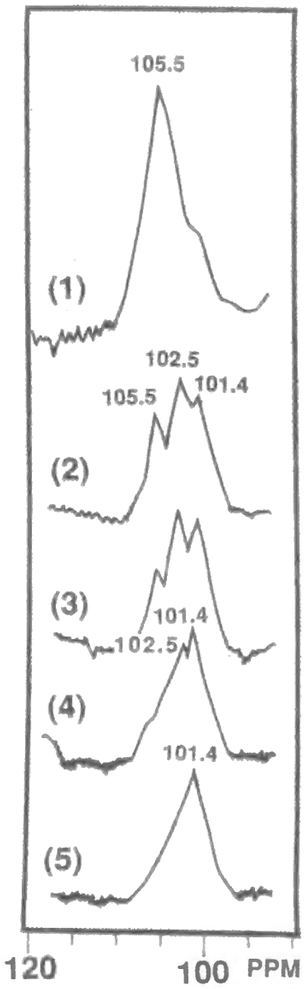


Figure 4-12. Change of the C-1 chemical shift of tritylcellulose and methylated tritylcellulose derivatives: (1) 6-*O*-tritylcellulose, (2) MTC1*, (3) MTC2*, (4) MTC3* and (5) 2,3-di-*O*-methyl-6-*O*-tritylcellulose.
* See Table 4-4.

When the methylation proceeded, the peak at 105.5 ppm decreased in intensity and the other two peaks appeared distinguishably. The peak at 102.5 ppm decreased and eventually the C-1 signal became one peak at 101.4 ppm in 2,3-di-*O*-methyl-6-*O*-tritylcellulose whose OH groups were completely blocked by methyl groups. This phenomenon also indicates that free OH at the C-2 position is rapidly methylated and then scission of the intramolecular hydrogen bonds at the C-2 position occurred and finally all OH groups at the C-2 position were completely methylated.

CONCLUSIONS

It is of importance to characterize hydrogen bonds formed in cellulosic molecules, for clarification of correlation between the structure and the physicochemical properties. Regioselectively substituted cellulose derivatives, 6-*O*- and 2,3-di-*O*-substituted cellulose derivatives were discussed in the light of hydrogen bonding formation. Rather broad OH frequencies in IR spectra of 2,3-di-*O*-substituted cellulose derivatives implied existence of intermolecular hydrogen bonds in addition to the intramolecular hydrogen bonds shown in Figure 4-3(B). Sharp and symmetric OH frequencies at 3465 cm^{-1} in IR spectra of 6-*O*-substituted cellulose derivatives indicated that two intramolecular hydrogen bonds between ether oxygen and OH group (O6---HO-2' and 3-OH---O5') form predominantly and strengths of the two bonds may be almost equivalent [Figure 4-3(C)]. Curve fitting analyses for the OH regions of the IR spectra supported the above hypothesis. Furthermore, CP/MAS ^{13}C-NMR spectra of the samples also supported the above although some question still remains. In addition, it was found that substituents at the C-6 position in 6-*O*-substituted cellulose derivatives had no significant influence on the formation of the two intramolecular hydrogen bonds. Only the introduction of the electron-withdrawing and bulky trityl group at the C-6 position changed the formation of the hydrogen bonds to possively give "free OH" appearing at 3580 cm^{-1} in the IR spectrum. The regioselectively substituted cellulose derivatives which may have controlled hydrogen bonds should

show characteristic physicochemical properties such as solubility, reactivity of OH groups and crystallinity compared with conventional cellulose derivatives. The characterization of the physicochemical properties of the polymers will be described in the next chapter.

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Chapter 5

Relationship between Intramolecular Hydrogen Bonds and Certain Physicochemical Properties of Regioselectively Substituted Cellulose Derivatives

ABSTRACT: This chapter tries to provide some direct evidence about the relationship between the intramolecular hydrogen bonds in cellulose and their corresponding effect on physicochemical properties. The formation of intramolecular hydrogen bonds has been proved to contribute directly to certain physicochemical properties of cellulose, such as its solubility in solvents having different polarities, the relative reactivities of the hydroxyls in a repeating unit and its crystallinity, using a 6-*O*-methylcellulose (6-*O*-MC) film that is expected to have intramolecular hydrogen bonds. The excellent solubility of 6-*O*-MC when compared with other cellulose derivatives indicates a lack of intermolecular hydrogen bonds. A comparison of the relative reactivities between the hydroxyls at the C-2 and C-3 position in 6-*O*-MC also indicates that intramolecular hydrogen bonds once formed in 6-*O*-MC films are possibly maintained even after dissolution in solvents. In addition, the poor crystallinity exhibited by 6-*O*-MC supports the idea that crystallization in cellulosics may be dependent more upon preferential intermolecular hydrogen bonding at the OH groups at the C-6 position than upon a uniform structure such as that found in 6-*O*-MC, where every structural unit is completely and regioselectively substituted, distinguishing it from other synthetic polymers such as polyolefins and polyesters.

INTRODUCTION

We have to date focused on clarifying the hydrogen bonding formation involved in cellulose molecules in various states, such as amorphous miscible blend films, gels, amorphous films and liquid crystalline phases[1-7], by using regioselectively substituted methylcelluloses like 2,3-di-*O*-methylcellulose (2,3-di-*O*-MC)[8] and 6-*O*-methylcellulose (6-*O*-MC)[9] as cellulose model compounds. These investigations were performed mainly by using tools such as FT-IR, DSC, fluorescence spectroscopy and solid state NMR. Interestingly, the OH absorption band in IR spectrum of 6-*O*-MC films was sharper and more symmetric than that for 2,3-di-*O*-MC which has only one OH group per anhydroglucose unit. This phenomenon was also observed for other substituents with longer alkyl, benzyl and allyl groups. To clarify this behavior, this author has proposed a model to explain the formation of intramolecular hydrogen bonds in 6-*O*-substituted cellulose derivatives. Specifically, these cellulose derivatives are expected to have two intramolecular hydrogen bonds, one between the OH at the C-3 position and an adjacent ether oxygen of the glucose ring, and the second between the ether oxygen at the substituted C-6 position and an adjacent OH at the C-2 position. FT-IR and CP/MAS ^{13}C-NMR measurements[1] provided evidence for the existence of these two types of intramolecular bonds. Accordingly, the aggregation state of 6-*O*-substituted compounds should be interesting to study particularly in terms of any relationship between the formation of the hydrogen bonds and their direct effect on certain physicochemical properties.

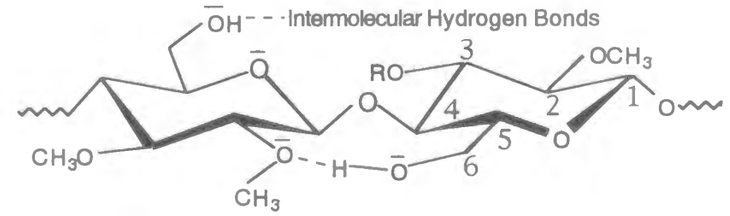
In general the formation of inter- and intramolecular hydrogen bonds in cellulose and its derivatives is considered to have a strong influence on their physicochemical properties. However, only a few published reports[10-13] have dealt directly with the above point of view, although many papers[14,15] have referred indirectly to this relationship between structure and physicochemical properties. Therefore, in this chapter, one 6-*O*-substituted cellulose ether, namely 6-*O*-methylcellulose, which has been revealed to have mainly intramolecular hydrogen bonds, has been chosen and its physical properties including solubility, hydroxyl reactivity and crystallinity, which should be influenced directly by any intramolecular hydrogen bonds, are investigated.

EXPERIMENTAL

Materials

The cellulose model samples, 2,3-di-*O*-MC and 6-*O*-MC (Figure 5-1(a) and 5-1(b), respectively) were prepared following previous methods[8,9]. Each polymer had a uniform structure in which every structural unit was regioselectively and completely substituted and the average degree of polymerization was approximately 120 on the basis of SEC elution curves calibrated with polystyrene standards. Powdered 2,3-di-*O*-MC and 6-*O*-MC as prepared were used for solubility tests.

(a) 23MC: Intra; OH at C-6 and OCH_3 at C-2.
Inter; OH at C-6.



(b) 6MC: Intra; OH at C-3 and O in the neighboring ring, OH at C-2 and OCH_3 at C-6.
Inter; NONE.

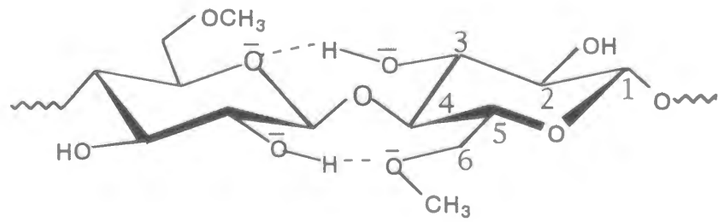


Figure 5-1. Types of hydrogen bonds possible in 2,3-di-*O*-MC (a) and 6-*O*-MC (b).

Film samples cast from both DMAc and a mixture of methanol/chloroform (1/3 v/v) were used for other experiments. Three kinds of methylcelluloses with different degrees of substitution (DS) were used as standards and are referred to as "Commercial MC" in Table 5-1. They were prepared in aqueous media following a commercial procedure, so that the substituent would be randomly distributed along the molecular chain.

Solubility

The solubility of 2,3-di-*O*-MC and 6-*O*-MC in various solvents was judged by examining whether a suspension containing 1.0% of the solute became transparent or not by visual inspection, in the temperature range between 20 and 50°C after two hours of constant stirring.

Relative Reactivities of the Remaining OH Groups at the C-2 and C-3 Positions

The chemical reactivities of the unreacted OH groups in the 6-*O*-MC, 6-*O*-tritylcellulose (6-*O*-TC) and 6-*O*-benzylcellulose (6-*O*-BC) were compared with one another. Figure 5-2 outlines schematically the analytical procedure followed: methylation in a homogeneous DMSO solution was used as a standard reaction for the OH groups[8]. One gram of each of the three samples in film form was dissolved completely in 60ml of DMSO at 50°C and pulverized NaOH powder, corresponding to 10 molar equivalents for each OH group, was added to the solution at room temperature with constant stirring for an hour. An equivalent mol of methyl iodide was then added dropwise over 30 minutes, the temperature was raised to 70°C and the solution mixtures were allowed to continue reacting for 4 hours. After the reaction was complete, the solution mixtures were poured into 95%(v/v) aqueous ethanol and the methylated products were precipitated. Filtration and the purification procedure[8] resulted in three kinds of the methylated 6-*O*-substituted cellulose derivatives, 6MC-1, M-TC-1 and M-BC-1 being prepared from 6-*O*-MC, 6-*O*-TC and 6-*O*-BC, respectively.

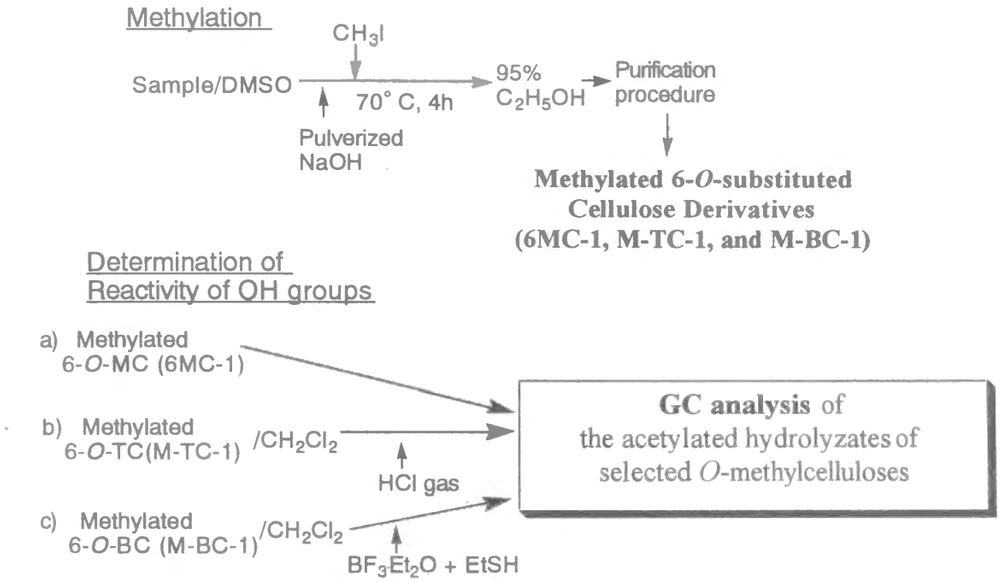


Figure 5-2 Schematic procedure for a gas chromatographic analysis to determine the relative reactivities of the hydroxyls in 6-*O*-MC, 6-*O*-TC, and 6-*O*-BC.

The relative reactivity for each OH group was determined by the distribution of methyl groups in a repeating unit based on a gas-chromatographic analysis of acetylated hydrolyzates of the polymers[8,9,16]. Methylated 6-*O*-MC (6MC-1) was used for the hydrolysis without further treatment. Trityl groups in the M-TC-1 were removed in CH_2Cl_2 with HCl gas bubbling at room temperature[8,9]. After the resultant methylated cellulose was isolated and purified as previously described, it was used for the hydrolysis. As for the M-BC-1, following debenzylation with a mixture of boron trifluoride etherate ($BF_3 \cdot Et_2O$) and ethanethiol (EtSH) in CH_2Cl_2[9,17], it was purified[9] and was then hydrolyzed. These acid-hydrolyzates were subjected to reduction with $NaBH_4$ and subsequent acetylation, and then injected into the gas-chromatograph.

Crystallinity

Powdered 6-*O*-MC and 2,3-di-*O*-MC were dissolved in DMAc and a mixed $CHCl_3/CH_3OH$ (4/1 v/v) solvent and films were cast at 50°C for the DMAc solutions and at room temperature for the $CHCl_3/CH_3OH$ solutions. The as-cast films (20 μm thick) were then dried under vacuum at 50°C for 7 days to ensure complete removal of all solvents. To confirm the complete removal of the solvents, the films were washed thoroughly with water and vacuum-dried, and then were compared with the as-cast films by FT-IR spectroscopy. It proved that in the as-cast films no solvent was trapped. The dried films were then subjected to X-ray diffraction measurements using JEOL X-ray diffractometer (model JDX-8200). Non-crystalline cellulose films cast from the LiCl-DMAc solution[6] and the SO_2-diethyl amine-DMSO solution[18] were prepared as previously described[6] and were used as references.

RESULTS AND DISCUSSION

As shown in Figure 5-1(b), 6-*O*-MC is a typical cellulose derivative which is expected to have two intramolecular hydrogen bonds shown by FT-IR analyses[1]; one between the OH at the C-3 position and its neighboring ring oxygen (O-5) and the second between the OH at the C-2 position and the ether oxygen of OCH_3 at the C-6 position. Therefore, 6-*O*-MC was deemed ideal to investigate the relationship between intramolecular hydrogen bonds and the following physicochemical properties which are closely related to one another. Three typical properties chosen to study here were the solubility, the relative reactivities of the remaining OH groups at the C-2 and C-3 positions in a repeating unit, and the crystallinity. The author chose these properties because he wanted to answer the following three questions: i) can the polymer be easily dissolved in various solvents due to a lack of intermolecular hydrogen bonds, ii) is it possible to maintain intramolecular hydrogen bonds after dissolution to allow the solution to have an influence on the OH reactivities

mentioned above and iii) is the solution less likely to crystallize than other ordinary cellulose and cellulose derivatives because of a restriction in the intermolecular hydrogen bonding which is available to the crystallizing polymer.

Table 5-1. Solubilities of various *O*- methylcelluloses (MCs).

Solvent	Commercial MC			2,3-di-O-MC	6-O-MC
	DS 0.1-1.1	DS 1.4-2.0	DS 2.4-2.8	DS 2.0	DS 1.0
Water	△	○	×	×	×
Aq. Alkali (pH 9.5)	○	△	×	△	△
Aq. Acid (pH 5.5)	○	○	△	○	○△
Acetone	×	×	×	×	○
Methanol	×	×	×	△	○
THF	×	×	○	×	○
Chloroform	×	×	○	△	○
DMSO	○△	○	○△	○	○
DMAc	○△	○	○△	○	○

O: Soluble, △: Swelling, ×: Insoluble, ○△: Partially soluble.

Solubility

Table 5-1 lists the solubilities of *O*-methylcelluloses; 2,3-di-*O*-MC, 6-*O*-MC and three kinds of *O*-methylcelluloses prepared commercially in an aqueous system so as to have different DS. In general, the higher the DS the higher solubility for cellulose derivatives. However, interestingly enough, 6-*O*-MC having a lower DS showed a higher solubility than 2,3-di-*O*-MC with a DS of 2.0. As shown in Chapter 4[1], the FT-IR spectra for 2,3-di-*O*-MC and 6-*O*-MC showed that this was due to the presence of intermolecular hydrogen bonds at the C-6 position of 2,3-di-*O*-MC. Further in comparing both 2,3-di-*O*-MC with a DS of 2.0 and 6-*O*-MC with a DS of 1.0 with other *O*-methylcelluloses having corresponding DS, respectively, the solubility of both 2,3-di-*O*-MC and 6-*O*-MC exhibited remarkable differences. In particular, 6-*O*-MC which is expected to have mainly intramolecular, rather than intermolecular, hydrogen bonds exhibited more favorable solubility in solvents having different polarities. These results indicate that the solubility of 6-*O*-MC is mostly determined

by the absence of intermolecular hydrogen bonds which can obstruct swelling and solvation of the polymer. As is already reported in previous papers[3,6,7], primary OH at the C-6 position can favorably form intermolecular hydrogen bonds, causing poor solubilities of materials to solvents. In other words, the amount of free OH groups at the C-6 position may contribute more critically to determining solubilities of the materials than that of free OH groups at the C-2 or C-3 position. In fact, comparing 6-*O*-MC with MC of DS2.4-2.8 in Table 5-1, primary OH at the C-6 position was fully substituted for 6-*O*-MC whereas for the MC with DS2.4-2.8 unsubstituted primary OH at the C-6 position was more or less remaining. Therefore, it may cause the superior solubility for 6-*O*-MC to the MC with 2.4-2.8 although total DS for the latter MC is higher.

Relative Reactivities of the Remaining OH Groups at the C-2 and C-3 Positions

Concerning the difference in relative reactivity of the OH groups at the two (C-2), three (C-3) and six (C-6) positions (OH-2, OH-3, and OH-6, respectively) of the anhydroglucose unit, it is well known[19,20] that the order of the relative reactivities is OH-2 > OH-6 > OH-3 for MC prepared from alkali-cellulose in an aqueous solvent system. The higher reactivity of OH-2 has been postulated to be due to its high acidity which is enhanced by its proximity of the anomeric center, C-1[21]. Therefore, if an intramolecular hydrogen bond was still present at the C-2 position even in the reaction mixuture, then the reactivity of OH-2 should be altered, and it would probably be reduced. Within this definition, the relative reactivities of the remaining hydroxyl groups (OH-2 and OH-3) in 6-*O*-MC, 6-*O*-TC, and 6-*O*-BC were examined. As reported previously[1] the two kinds of 6-substituted derivatives, 6-*O*-TC and 6-*O*-BC are assumed not to have any intramolecular hydrogen bonds, only 6-*O*-MC has them.

Table 5-2 shows the results of a gas-chromatographic analysis for the distribution of methyl group (X_n; n=2, 3, and 6) on the three MC samples. X_2, X_3, and X_6 were calculated from molar ratios of glucitol (S_0) and its mono-, di- and trimethyl

Table 5-2. Distribution of methyl groups in three methylated 6-*O*- substituted cellulose derivatives.

Sample	Overall DS	Molar Ratios (%)								DS at Positions		
		S_0	S_2	S_3	S_6	S_{23}	S_{36}	S_{26}	S_{236}	X_2	X_3	X_6
6MC-1	2.0	0	0	0	34	0	20	15	31	0.46	0.51	1.0
M-TC-1	1.3	14	32	13	0	42	0	0	0	0.74	0.55	0
M-BC-1	1.1	21	41	12	0	26	0	0	0	0.67	0.38	0

Overall DS equals $X_2+X_3+X_6$. Each Sn was determined by a gas-chromatographic analysis.

Xn is the mol fraction of glucitol derivatives substituted on the OH of C-n(n=2,3, and 6).

For example, $X_2=(S_2+S_{23}+S_{26}+S_{236})/100$.

derivatives (S_2-S_{236}), which were derived from the hydrolyzates of the polymers as determined by gas-chromatography:

$$X_2 = S_2 + S_{23} + S_{26} + S_{236}$$
$$X_3 = S_3 + S_{23} + S_{36} + S_{236}$$
$$X_6 = S_6 + S_{26} + S_{36} + S_{236}$$
$$DS = X_2 + X_3 + X_6$$

These X_n values show the reactivities for each OH group, as well as the distribution of the methyl substituents at the OH group in each position of the carbons (C-2, C-3, and C-6). The DS represents the sum of X_2, X_3, and X_6. From the DS values at the C-2 and C-3, ($X_2 + X_3$), the relative reactivities were found to be in the following order: 6-*O*-TC > 6-*O*-BC > 6-*O*-MC. This is apparently due to the difference in reactivity of the OH group at the C-2 position. Moreover, this difference may be directly attributed to the presence of intramolecular hydrogen bonds at the C-2 position. As reported earlier by a FTIR analysis[1] in Chapter 4, the introduction of electron-withdrawing and bulky functional groups such as trityl and benzyl groups at the C-6 position changes the structure of the intramolecular hydrogen bonds to give free OH groups at the C-2 position and, in addition, the substituent effect of the more bulky trityl group shows itself more in the appearance of free OH-2 than in the smaller benzyl group. Further, the bulkiness of the trityl groups at the C-6 position for 6-*O*-TC may change the conformation of the glucose backbone to cause, to some extent, a break of the intramolecular hydrogen bonds between the OH at the C-3 position and its neighboring ring oxygen (O-5). Thus, these produced free OH-3 for 6-*O*-TC can be more methylated than 6-*O*-BC as shown in Table 5-2. In the case of 6-*O*-MC, this deformation of the intramolecular hydrogen bonds is not expected by the substitution at the C-6 position. Therefore, the order of 6-*O*-TC > 6-*O*-BC > 6-*O*-MC above can be considered as the reverse order of preference for the formation of intramolecular hydrogen bonds. Indeed, in 6-*O*-MC which is expected to have strong

intramolecular hydrogen bonds, the relative reactivities at both the C-2 and C-3 positions exhibited almost the same values, as was distinctly different for both 6-*O*-TC and 6-*O*-BC. Judging from the X_2 and X_3 values, this does not mean an enhanced reactivity of the OH-3 reactivity, but rather a reduction in the OH-2 reactivity probably due to the formation of intramolecular hydrogen bonds at this position. Simultaneously, the hydrogen bonds at the C-2 position which form between OH-2 and the ether oxygen of a methoxy group at the C-6 position seems to be very similar to the intramolecular hydrogen bonds at the C-3 position, between OH-3 and the glucose ring oxygen. Therefore, reactivities at both the C-2 and C-3 positions show similar values. In contrast, 6-*O*-TC and 6-*O*-BC exhibited relative reactivities in the order of OH-2 > OH-3. Taking hydrogen bonding into account, this order is reasonable and it coincides with that usually exhibited in aqueous systems as mentioned previously. In this study, it is noted that the methylation was performed in homogeneous DMSO solution and therefore, the hydroxyl groups in 6-*O*-TC and 6-*O*-BC may be solvated to prevent further involvement of the hydrogen bonds. In 6-*O*-MC, as stated above, the relative reactivities at the C-2 and C-3 positions indicate that the intramolecular bonds might be still maintained even in the homogeneous solution state.

Crystallinity

As shown in Figure 5-3(a), the X-ray diffraction patterns for 6-*O*-MC films cast from both DMAc and $CHCl_3/CH_3OH$(4/1 v/v) solvents exhibited low crystalline patterns similar to those obtained from amorphous celluloses obtained from the SO_2-diethylamine-DMSO solutions [Figure 5-3 (e)], and further the 6-*O*-MC did not show a crystalline pattern even after heat treatment at 160°C. Thus, 6-*O*-MC shows poor crystallinity irrespective of its homogeneity and regularity of the structural unit along a molecular chain. In general, crystallization depends not only on regularity of chemical structure but also on sufficient chain flexibility for coordinated molecular motion to form nuclei. The 6-*O*-MC chain may be sufficiently stiff to form a high

viscosity medium during evaporation of the solvent which would prevent nucleation. Therefore, precipitation/crystallization in a dilute solution was tried. However, crystallized 6-*O*-MC could not be obtained after this process. Another possible reason

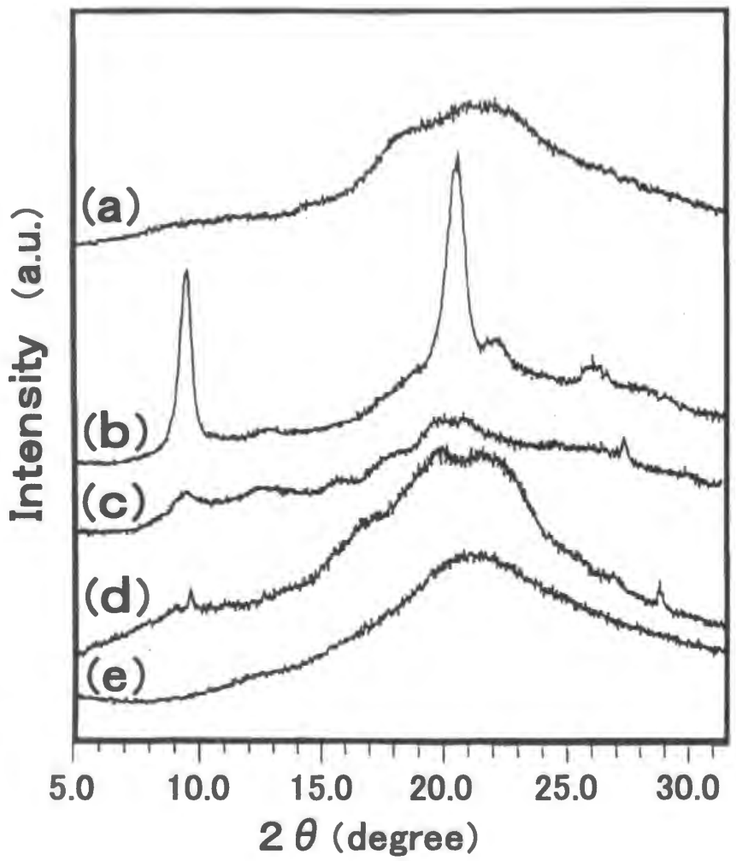


Figure 5-3. Wide-angle X-ray diffraction patterns for: (a) 6-*O*-MC cast films; (b) 2,3-di-*O*-MC film cast from DMAc solution; (c) 2,3-di-*O*-MC film cast from $CHCl_3/CH_3OH$ (4/1 v/v) solution; (d) cellulose prepared from the DMAc/LiCl solution; (e)amorphous cellulose prepared from the SO_2-diethylamine-DMSO solution.
The a.u. indicates arbitrary unit.

why the X-ray diffraction pattern for 6-*O*-MC exhibited a low crystallinity is that the crystalline size may be very small or the crystals may be hard to be grown, if any. On the other hand, 2,3-di-*O*-MC showed different patterns depending on the solvents as shown in the patterns (b) and (c) of Figure 5-3. As indicated in papers on crystallization[6] and gel formation[3] (Chapter 7) of cellulosics, the primary OH groups at the C-6 position may be favorably involved in intermolecular hydrogen bonding. The extent of crystallization may depend on the behavior of the primary OH groups. Thus, cellulose derivatives whose OH groups at the C-6 position are blocked like in 6-*O*-MC may prevent crystallization in the same manner as crystallization resulting from intermolecular hydrogen bonding in say 2,3-di-*O*-MC. Stated differently, 6-*O*-MC which shows strong intramolecular hydrogen bonds can perhaps possibly form a crystalline state simply from van der Waals force by a minimization of the system potential energy. However, the poor crystallinity exhibited by 6-*O*-MC described above suggests that intermolecular hydrogen bonds at the OH groups at the C-6 position may be more advantageous in aiding crystallization in cellulosics than van der Waals force.

Concerning precedence for the primary OH at the C-6 position to the secondary OH at the C-2 and C-3 positions in crystallization, one reason is that as described above 2,3-di-*O*-MC which has only free primary OH at the C-6 position was easier to be crystallized than 6-*O*-MC and yet the crystallized 2,3-di-*O*-MC did not show a melting point. This appears probably due to the strong intermolecular hydrogen bonding engagements in the crystallized 2,3-di-*O*-MC. Such crystallization accompanied by engagements of the hydrogen bonds for cellulosic materials might, more or less, depend on a favorable formation of the intermolecular hydrogen bonds between OH at the C-3 position and OH at the C-6 position as suggested by Gardner and Blackwell[23], and Sarko and Muggli[24]. As for the favorability of the interaction, The author has already reported[2] that in comparison of 2,3-di-*O*-MC with 6-*O*-MC for the blend with PEO which has oxygen in the polymer backbone, only primary OH

at the C-6 position for 2,3-di-*O*-MC was engaged in intermolecular hydrogen bonds whereas secondary OH at the C-2 or C-3 position for 6-*O*-MC did not form the interaction with PEO. Therefore it is considered that the primary OH at the C-6 position may contribute to the crystallization more than the secondary OH.

CONCLUSIONS

The physicochemical properties of cellulose and its deivatives are strongly influenced by the formation of inter- and intramolecular hydrogen bonds. In this chapter the author has attempted to provide direct evidence for the relationship between intramolecular hydrogen bonds and their influence on the physicochemical properties of the polymer. Since 6-*O*-methylcellulose had been shown to have only intramolecular hydrogen bonds as illustrated in Figure 5-1(b), the author examined in greater detail the physicochemical properties of this polymer. As typical properties, solubility, relative reactivity for the remaining OH groups at the C-2 and C-3 positions in a repeating unit and crystallinity were chosen. The conclusions drawn are shown schematically in Figure 5-4: The excellent solubility of 6-*O*-MC in various different solvents was attributed to the absence of intermolecular hydrogen bonds in 6-*O*-MC, which was proposed in Chapter 4[1]. The similar relative reactivities at both OH-2 and OH-3 in 6-*O*-MC, in contrast to other cellulose derivatives, indicates that intramolecular hydrogen bonds may possibly be maintained even after solvation to allow solution. The poor crystallinity exhibited by 6-*O*-MC also supports our previous suggestion[3,6] that the usually observed crystallization in cellulose may be dependent on the ability of the OH groups at the C-6 position to engage in intermolecular hydrogen bonding. In other words, the fact that the uniform structure of 6-*O*-MC in which every structural unit is completely and regioselectively substituted can engage in intramolecular hydrogen bonds, is not an advantage for crystallization, which differs from other synthetic polymers such as polyolefins and

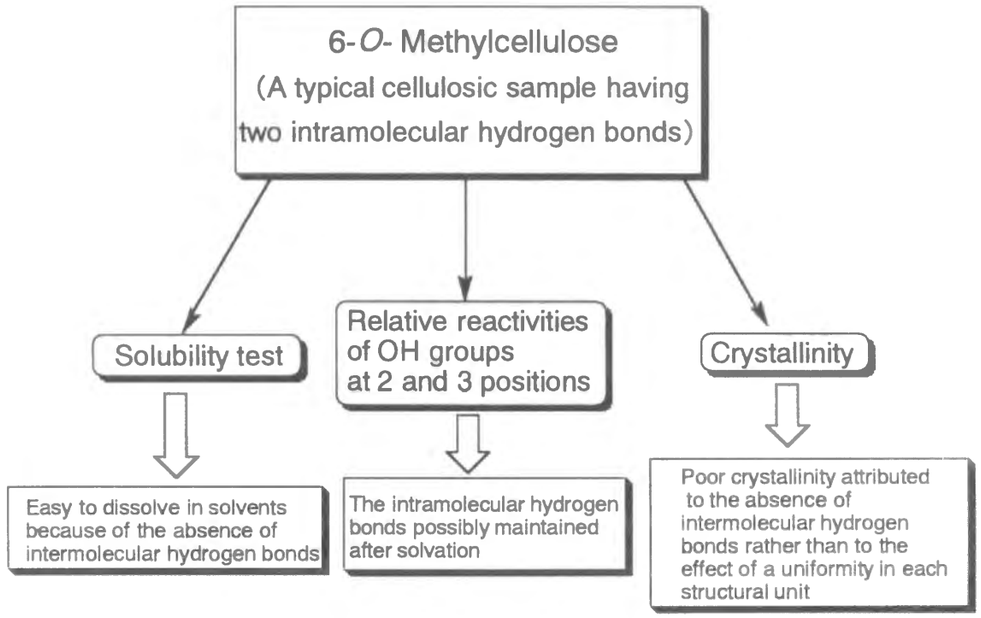


Figure 5-4. Schematic summary of the conclusions.

polyesters. This might be due to an induced stiffness in the main chain of 6-*O*-MC which result from the presence of intramolecular hydrogen bonds.

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Chapter 6

Influence of Intramolecular Hydrogen Bonds on Handedness in *O*-Ethylcellulose /CH_2Cl_2 Liquid Crystalline Mesophases

ABSTRACT: This chapter shows that intramolecular hydrogen bonds formed between the OH groups contribute in determining the handedness of chiral nematic mesophases *O*-ethylcellulose in CH_2Cl_2. Using two series of samples which differ only in the degree of ethylation of the OH groups at either the C-6 or C-3 positions, the handedness of mesophase solutions was investigated using circular dichroism (CD). The CD spectra indicated that free OH groups at the C-6 position hinder dissolution in the solvent while free OH groups at the C-3 position may play an important role in determining the handedness of the EC mesophase in CH_2Cl_2. Free OH groups at the C-3 position are known to easily form intramolecular hydrogen bonds, resulting in enhanced stiffness of the molecular chains. Therefore, these results lead to the conclusion that the breaking of intramolecular hydrogen bonds by increasing the substitution along the molecular chain causes it to become more flexible, which may account for the change in structural handedness.

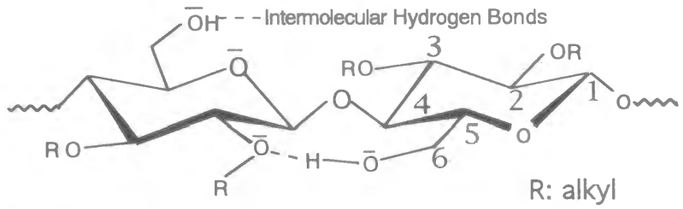
INTRODUCTION

In the previous chapters (4 and 5), the author has investigated the formation of inter- and intramolecular hydrogen bonds for cellulosic molecules in the non-crystalline films[1] using two types of cellulose model compounds, 2,3-di-*O*-

methylcellulose (2,3-di-*O*-MC) and 6-*O*-methylcellulose (6-*O*-MC[2,3]), which have regular structural units, i.e., regioselectively methylated glucopyranose units.

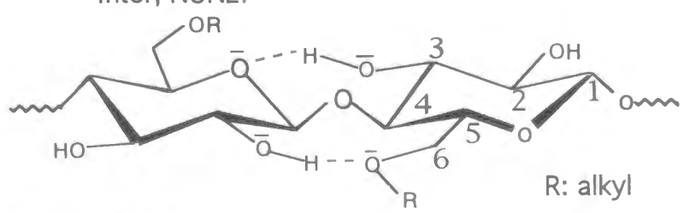
(a) 2,3-di-*O*- alkylcellulose:

Intra; OH at C-6 and OR at C-2.
Inter; OH at C-6.



(b) 6-*O*-alkylcellulose:

Intra; OH at C-3 and O in the neighboring ring,
OH at C-2 and OR at C-6.
Inter; NONE.



(c) Cellulose

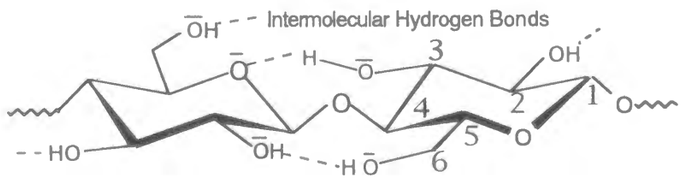


Figure 6-1. Possible hydrogen bonds formed at the individual C-2, C-3 and C-6 positions of the glucose ring in typical *O*-alkylcelluloses (a and b) and cellulose (c).

More specifically, 6-*O*-alkylcelluloses such as 6-*O*-MC mainly exhibit two kinds of intramolecular hydrogen bonds[1] as shown in Figure 6-1b: one type may form between the hydroxyls at the C-3 position and an adjacent ether oxygen of the glucose ring while a second type can form between the ether oxygen at the substituted C-6 position and an adjacent hydroxyl at the C-2 position. Further, in 6-*O*-MC samples there was a indirect correlation between the physicochemical properties observed and formation of the intramolecular hydrogen bonds which can be even maintained in solution[4] (See Chapter 5). The intramolecular hydrogen bonds were also found to have an influence on the enzymatic hydrolysis of 6-*O*-MC[5,6], which will be described in Chapter 8. We.must add that these intramolecular hydrogen bonds also exist, of course, in unmodified native cellulose as illustrated in Figure 6-1c.

Recently, the author succeeded in preparing a series of *O*-methyl- and *O*-ethylcelluloses having a systematically controlled distribution of substitutents and DS (degrees of substitution)[2]. These polymers would appear to form an ideal set of complete samples for determining what type of correlation exists between the physicochemical properties and the distribution of substituents for *O*-alkylcellulose derivatives in terms of hydrogen bonding formation. In this chapter, the author focused on the effect of the substituent distribution on the liquid crystalline properties of EC. Many cellulose derivatives form lyotropic and thermotropic liquid crystals which normally exhibit cholesteric or chiral nematic structures[7-13]. The majority of cellulosic mesophases that have been studied to date are right-handed cholesterics, but a few left-handed systems were also reported[14-19]. Specifically, lyotropic *O*-ethylcellulose (EC) mesophases were observed to show both types of handedness depending on the polymer volume fraction and solvent[14,15]. Guo and Gray[18,19] reported that cholesteric liquid crystalline solutions of acetylated EC in chloroform exhibit a change in handedness from a left-handed to a right-handed helicoidal supermolecular arrangement with an increasing acetyl content in the EC. The twist

sense of EC mesophases in chloroform and CH_2Cl_2 was also observed to change from left-handed to right-handed with an increasing degree of ethyl substitution in EC[20,21]. However, the driving force for this structure reversal is still not clear. In this chapter, the author will report on the relationship between hydrogen bonding formation and the reversal of handedness in EC mesophases in CH_2Cl_2. Using *O*-ethylcelluloses having a systematically controlled distribution of substitutents and DS as mentioned previously, the influence of intramolecular hydrogen bonds, within the same chiral backbone, in determining the handedness of these lyotropic mesophases has been investigated.

EXPERIMENTAL

Materials

Two series (A and B) of *O*-ethylcellulose samples were used in this experiment to cover the entire range of OH substitutions possible in EC.

Series A: Preparation from 2,3-Di-*O*-ethylcellulose (2,3-di-*O*-EC)

Series A consisted of 9 samples. The starting material with an average degree of polymerization (DP) of 200 was 2,3-di-*O*-ethylcellulose (2,3-di-*O*-EC), prepared as reported previously (Chapter 2)[2], having ethoxyl groups at both the C-2 and C-3 positions of the anhydroglucose unit (Figure 6-1a). The 2,3-di-*O*-EC was then ethylated 8 times to yield 8 different samples of 2,3-di-*O*-ethyl-6-*O*-partially ethylated cellulose having different DS at the C-6 position as schematically shown in Figure 6-2I. The 2,3-di-*O*-EC was added to 60 ml of DMSO with 2.0 g of pulverized NaOH powder and differing amounts of ethyl iodide. After 30 min of stirring under a nitrogen atmosphere at room temperature, the temperature was raised to 70°C and maintained there for 1 h for the first 5 samples and 3 h for the remaining three samples. The reaction mixture was then cooled to room temperature and poured into a 95% methanol solution. The resulting products were then precipitated and washed thoroughly with a 95% methanol solution and subsequently with distilled water. The

average yield for each preparation was approximately 85 to 90%. The final products had a DP of 200 indicating that significant depolymerization of the products did not occur during *O*-alkylation[2].

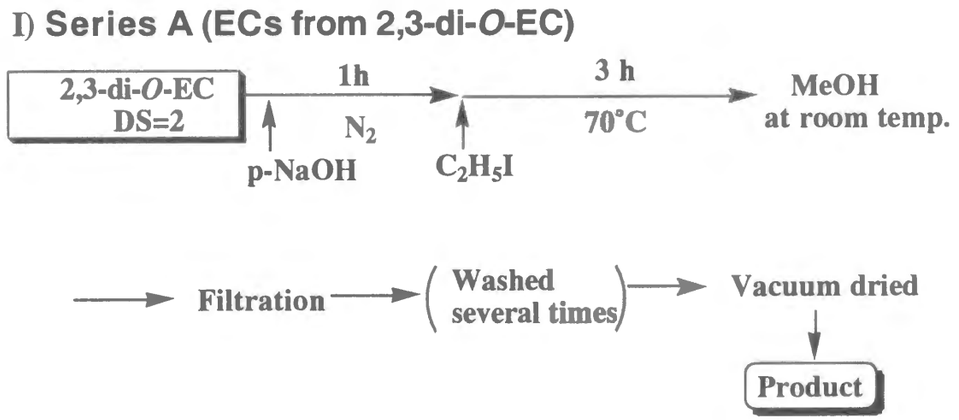


Figure 6-2. Preparation schemes for 2 series (A and B) of *O*-ethylcellulose samples with different distribution patterns for the ethyl substituents.

Series B: Preparation from a Commercial *O*-Ethylcellulose

This second series of samples were prepared following the Budgell method[20,21], as shown in Figure 6-2II. The starting material was a commercial EC obtained from Aldrich Ltd and had a DS of 2.3 and a mass average molar mass of 65,000. Dried EC, 5 g, was dissolved in 60 ml of tetrahydrofuran under nitrogen. Sodium hydride (NaH), 0.7 g, was then added and the solution was stirred for 1 h before the dropwise addition of ethyl iodide as the alkylating agent. The temperature ranged from 40 to 60°C, the reaction time and the amount of ethyl iodide added were varied to give samples with ethyl DS of 2.3 to 3.0. After cooling, 50 ml of methanol were slowly added to the reaction mixture to remove any unreacted NaH and the polymer was precipitated by pouring into 200 ml of water containing 1 g of NaS_2O_3. It was then thoroughly washed with water and dried under vacuum at 60°C. Degradation of the polymer during the ethylation was not severe since the samples had molar masses similar to that of the starting EC.

Characterization of the Distribution of Ethyl Substituents

The distribution of ethyl groups in the EC samples was determined by acid hydrolysis and subsequent analysis of the components as partially ethylated alditol acetates by gas-liquid chromatography[2,4]: The samples were treated with 4% sulfuric acid at 120 °C for 1 h. Following neutralization to pH 5.5, the acid-hydrolyzates were subjected to reduction with $NaBH_4$ for overnight and dried completely for the subsequent acetylation with acetic anhydride. Then the alditol acetates in acetone were injected into the gas-chromatograph.

Analyses

Thin samples of EC and dichlorometane (CH_2Cl_2) were prepared for optical measurements by flame-sealing one end of a 0.4 mm thick glass microslide (Vitro Dynamics Inc). The slide was packed with a known mass of polymer and

predetermined mass of solvent was injected with a microsyringe. The sample was then cooled in liquid nitrogen and the other end of the microslide was flame-sealed. CD measurements were performed on polymer concentrations ranging from 40 to 50 wt% where the EC solutions in CH_2Cl_2 formed chiral nematic mesophases exhibiting reflection colors. However, since CH_2Cl_2 is very volatile, the exact concentrations could not be determined with complete accuracy even using the above procedure. Centrifugation was used to promote mixing in the microslides. Then, the CD spectra were recorded by placing the microslides in the spectropolarimeter beam path.

Only qualitative analyses were possible because of the previously described concentration problems caused by the CH_2Cl_2 volatility and thus only the mesophase cholesteric twist sense was noted. The handedness and wavelength of the cholesteric reflection bands at 25°C were measured with a Jasco J-500C spectropolarimeter.

RESULTS AND DISCUSSION

Characterization of the Two Polymer Series

i) Series A

Figure 6-3 shows the change in DS for the ethyl groups at each position (C-2, C-3 and C-6 position) of the anhydroglucose unit for the entire set of 9 samples in the series A. These samples were prepared from 2,3-di-*O*-EC. For this set of samples, all OH groups at the C-2 and C-3 positions were almost completely ethylated and only the ethyl DS at the OH at the C-6 position was increased systematically with increasing sample code number. As already reported in a previous chapter (Chapter 4)[1,22,23], the OH groups at the C-6 position in cellulosics easily form intermolecular hydrogen bonds, resulting in poor solubility for cellulose and cellulose derivatives in many solvents. In the series A as seen in Figure 6-3, samples 1- 4 which had a DS at the C-6 position of less than 0.78 did not give clear CH_2Cl_2 solutions even at concentrations of 1 wt%. A high degree of substitution at the C-6 position was required for the polymer to dissolve in CH_2Cl_2.

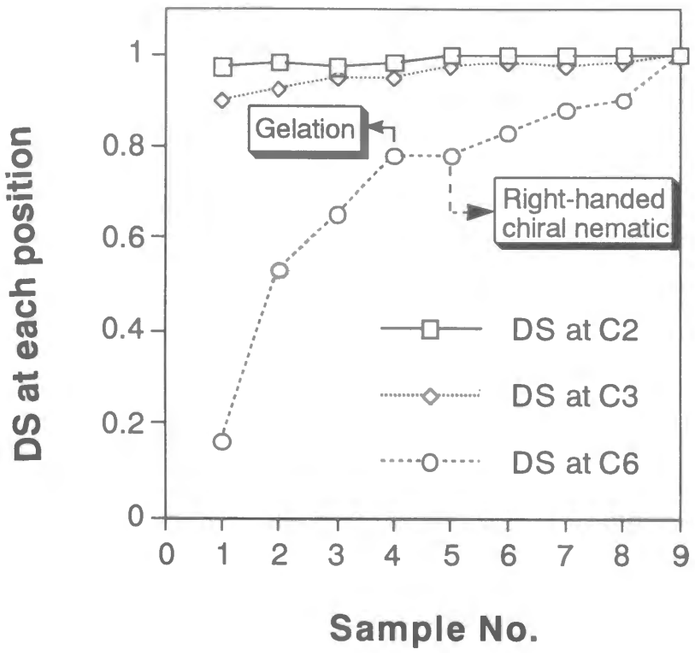


Figure 6-3. Change in the degree of substitution (DS) pattern at the individual C-2, C-3 and C-6 positions for series A samples prepared from synthesized 2,3-di-*O*-EC.

ii) Series B

These samples were prepared from a commercilally available EC. The distribution of ethyl substituents for the starting material (sample 1 in Figure 6-4) was 0.85, 0.6 and 0.85 for the C-2, C-3 and C-6 positions, respectively. The DS at the C-3 position was somewhat lower than that for the other positions. The OH groups at the C-2 and C-6 positions were easily and completely ethylated to give a saturated point with a DS of 1.0. Thus only the ethyl DS for the OH groups at the C-3 position was individually increased up to a limit of 1.0. In contrast to series A, the OH groups at

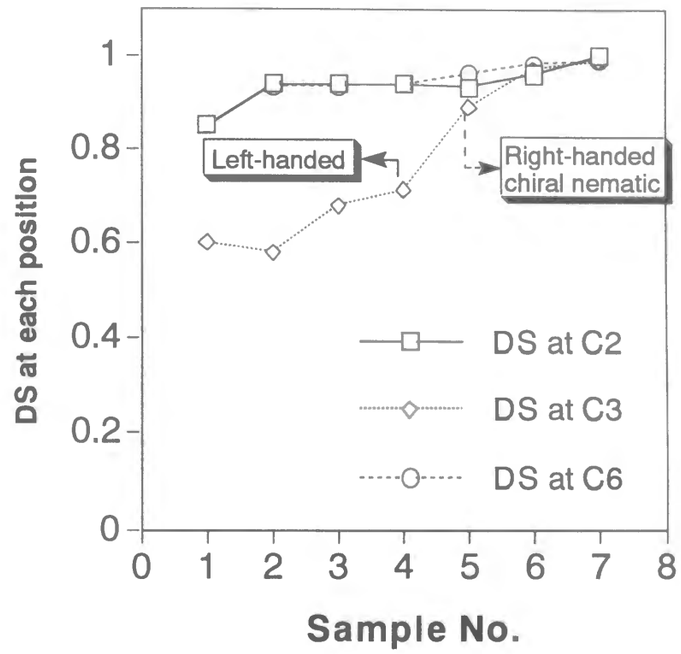


Figure 6-4. Change in degree of substitution (DS) pattern at the individual C-2, C-3 and C-6 positions for series B samples prepared from a commercially available EC.

the C-6 position in series B were almost completely ethylated and all samples dissolved in CH_2Cl_2 at 1 wt% to yield clear solutions.

Handedness of EC Lyotropic Liquid Crystals

Two of the primary optical methods for studying chiral nematic (cholesteric) liquid crystals are optical rotatory dispersion (ORD) and circular dichroism (CD). In this study, the author used only CD to determine the handedness of the cholesteric structure by the sign of the induced CD band which results from the selective reflection of circularly polarized light. A positive CD band corresponds to a left-

handed cholesteric twist, while a negative CD band corresponds to a right-handed twist.

Figure 6-5 shows the induced CD spectra for anisotropic CH_2Cl_2 solutions of EC 4, 5 and 7 in series B which has an increasing ethyl DS at the OH group at the C-3 position. The positive CD band for EC 4 indicates that the liquid crystal is left-handed while the negative CD bands for EC 5 and 7 indicate right-handed twists. The change in cholesteric handedness for all the series B samples is illustrated in Figure 6-4. The concentration range for the anisotropic phases was between 40% and 50% by weight of polymer. This suggests that the introduction of more ethyl groups into the OH group at the C-3 position leads to a change in the cholesteric handedness in this particular solvent, CH_2Cl_2.

Samples from series A, as shown in Figure 6-3, showed a totally different behavior in cholesteric handedness from that of series B samples. Series A samples which were prepared from 2,3-di-*O*-EC have free OH groups only at the C-6 position. As the sample code number increases, the free OHgroups at the C-6 position are gradually replaced by ethyl groups. As already noted, OH groups at the C-6 position in cellulosics contribute favorably to the formation of intermolecular hydrogen bonds and this results in poor solubility of the polymer. This can be seen for samples 1-4 in Figure 6-3 which do not dissolve completely even in very dilute solution. Instead, they were found to swell and form gels. Samples with a DS of more than 0.8 at the C-6 position, namely samples 5-9, show induced CD spectra for concentrated anisotropic solutions from 40 to 50 wt% polymer (Figure 6-3) and all are right-handed chiral nematic liquid crystals.

These results suggest strongly that the distribution of ethyl substituents among C-2, C-3, and C-6 positions of the anhydroglucose unit in EC samples can affect the cholesteric handedness of their anisotropic solutions. In particular, the substitution of OH groups at the C-3 position may play an important role in determining the handedness, while OH groups at the C-6 position are important and contribute to the solubility of the sample in various solvents. The author reported in Chapters 4 and 5

that the intramolecular hydrogen bonds formed between OH groups at the C-3 position and adjacent ether oxygens of the glucose ring (Figure 6-1b and -1c) are fairly strong[1,24] and may even be maintained in the solution state[4]. Further, it is recognized that intramoledular hydrogen bonds should have a strong influence on the physicochemical properties[4,25,26] of the polymer. In other words, intramolecular hydrogen bonds at the C-3 position may play a role in determining the conformation of the extended glucose chain structure causing greater chain stiffness. Therefore in the present study, once the OH groups at the C-3 position are highly substituted, then the intramolecular bonds must be cut (Figure 6-1a type), and the anhydroglucose unit is now freer and may rotate easily to alter the torsion angle between two consecutive units and as a result the molecular chain will be more flexible. In fact, T_1 relaxation time of the C-1 carbon in an anhydroglucose unit in cellulose derivatives whose hydroxyl groups at the C-3 position remains unsubstituted is longer than that for 2,3-*O*-substituted cellulosics even in the solution state.[13] ^{13}C-NMR chemical shifts and relaxation time at the C-1 carbon also change after deuteration of the OH groups in the same system[27]. These results indicates not only the engagements of the intramolecular hydrogen bonds in the solution state, but also the positive contribution of the interaction to the molecular chain stiffness. For all samples in series A, the OH groups at the C-3 position are assumed to be almost fully substituted and thus as long as the sample is dissolved, the anisotropic solution shows right-handedness. As shown in Figure 6-4 (series B), the samples with an ethyl DS more than 0.9 at the C-3 position, EC 5-9, form a lyotropic right-handed chiral nematic mesophase. In relating the intramolecular hydrogen bonds at the C-3 position with chain stiffness, the above results indicate that the less stiff the chain is, the more favorably the right-handed chiral nematic structure is formed.

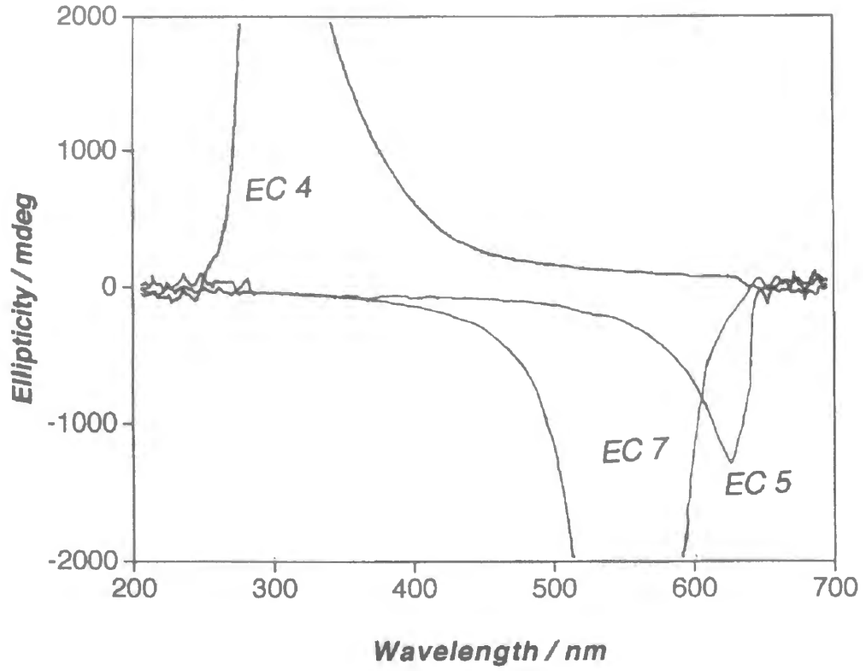


Figure 6-5. Induced CD spectra for mesophase of EC 4, EC 5 and EC 7 (series B) in CH_2Cl_2 at 25°C.

In series B samples, as the polymer concentration was increased from an isotropic solution to a film, there was no appearance of visible reflection colors or of liquid crystalline fingerprint textures as viewed under cross polarizers in the microscope. This suggests that the mesophase is in the untwisted nematic state[20,21]. As shown in Figure 6-6, both left-handed (EC 1) and right-handed (EC 8) mesophases in CH_2Cl_2 show similar behavior in their relationship between the reflection wavelength and the concentration; the lower the polymer concentration, the longer the reflection wavelength. For planar chiral nematic liquid crystals the pitch, *P*, of the supermolecular helicoidal assembly of molecular chains is related to the maximum

reflection wavelength, λ_0, by de Vries equation[28], $\lambda_0 = nP$, where n is the average refractive index of the mesophase. Therefore, the concentration dependence trend is explained by the decrease in pitch of the cholesteric structure with increasing polymer concentration irrespective of the handedness. Unfortunately the author could not examine the temperature dependence of the reflection wavelength because of experimental difficulties in the system at present.

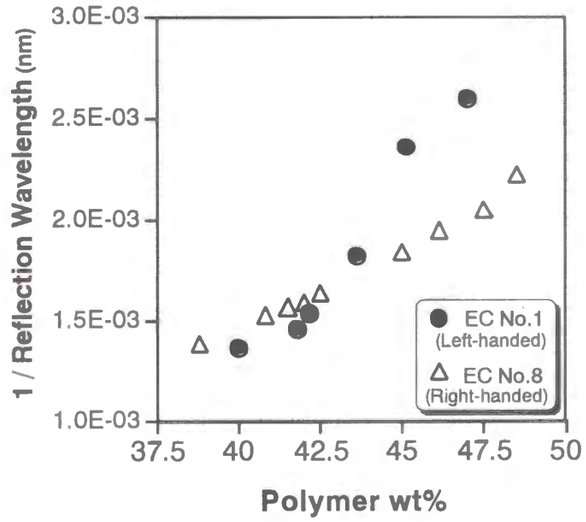


Figure 6-6. Dependence of maxmum reflection wavelength on concentration for left- and right-handed mesophases of EC (series B EC 1 and EC 8, respectively) in CH_2Cl_2 at 25°C.

CONCLUSIONS

Using two series of samples, in which degree of substitution (DS) at the individual hydroxyl groups at the C-2, C-3 and C-6 positions of the glucose unit were

systematically changed, the dependence of the chiral nematic handedness of EC mesophases on the regioselectivity of the DS was investigated. As already reported in previous chapter (Chapter 5)[4,22,23], free OH groups at the C-6 position interfered with the solubility of the samples in CH_2Cl_2. When the OH groups at the C-3 position were highly substituted by ethyl groups, the mesophase in CH_2Cl_2 exhibited a right-handed twist. Samples with a DS of less than 0.71 at the C-3 position in CH_2Cl_2 formed left-handed mesophases with reflection colors. The OH groups at the C-3 position are known to easily form intramolecular hydrogen bonds with neighboring ring oxygens. Therefore, it is concluded that intramolecular hydrogen bonds formed between the OH groups at the C-3 position and the O-5 position oxygens may have a strong influence on the handedness of the cholesteric or chiral nematic supermolecular arrangement in EC. In addition, as chain stiffness arising from the intramolecular hydrogen bonds decreases a twist reversal for the mesophase can occur - specifically it was found that as the DS of the EC increases samples with flexible chains caused by high ethyl substitution at the C-3 position (Figure 6-1a type) exhibited right-handed supermolecular arrangement in CH_2Cl_2, while left-handed mesophases of EC were found in samples with lower DS where rather stiff molecular chains had more intramolecular hydrogen bonds. These results indicate that the contribution of intramolecular hydrogen bonds should be considered when trying to explain or account for the chiroptical properties of cellulosic mesophases.

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Chapter 7

Physical Gelation Process for Cellulose Derivatives Whose Hydroxyl Groups Are Regioselectively Substituted by Fluorescent Groups

ABSTRACT: Five cellulose derivatives whose OH groups are regioselectively substituted by benzyl ether and methyl ether groups were preprared; i.e., 6-*O*-benzylcellulose, 2,3-di-*O*-methyl-6-*O*-benzylcellulose, 2,3-di-*O*-benzylcellulose (2,3-BC), 2,3-di-*O*-benzyl-6-*O*-methylcellulose, 2,3,6-tri-*O*-benzylcellulose. The gelation did not take place in tetrahydrofuran (THF) solutions of the cellulose derivatives whose OH groups at the C-6 position were substituted to methyl ether or benzyl ether groups, but the derivative having free OH groups at the C-6 position, i.e., 2,3-BC, was found to form gels in THF. All the samples except 2,3-BC showed only the usual fluorescence of benzyl group over the temperature range of 200-310K. In contrast, the fluorescence of 2,3-BC shifted to the red and the excimer fluorescence increased with an increase of interactions between the cellulose molecules. The present method using fluorescent probe was found to be very useful to elucidate the gelation process with a change of temperature in terms of the molecular association involving hydrogen bonds. In addition, the new absorption corresponding to the red-shifted fluorescence peak was also confirmed. The new species were concluded to be a ground state dimer formed intermolecularly between benzyl groups, indicating that there exists a hydrophobic interaction between them. In conclusion, (1) the main cause for the gel formation in the systems examined is the hydrogen bonding due to the OH groups at the C-6 position and (2) in addition to hydrogen bonding, the hydrophobic interaction between benzyl groups also keeps 2,3-BC molecules associated with one another after it is aggregated.

INTRODUCTION

The characteristic features of cellulose molecules such as the semi-rigidity of the chain and the insolubility in many solvents are attributed to the presence of three OH groups at the C-2, C-3, and C-6 positions in the anhydroglucose units forming both inter- and intramolecular hydrogen bonds. The substitution of the OH groups by functional groups is known to induce gelation[1] or liquid crystallization[2-4], where the inter- and intramolecular hydrogen bonds may play an important role. Thus, the author has aimed at elucidating the function of each OH group of cellulose in aggregated states such as gels.

Itagaki *et al.* have succeeded in studying the gelation process of isotactic polystyrene in decalin using the fluorescent probe method,[5,6] since luminescent probe techniques can be effective tools for investigating microstructures and motions of polymer molecules, especially because of their having the advantage of high sensitivity.[7] However, fluorescence measurements have not been so effectively employed in investigating microstructures and motions of cellulose molecules thus far except for the work by Winnik and co-workers[8-12], who introduced pyrenyl groups into hydroxypropylcellulose as a fluorescent probe and monitored their emission behaviour in relation to polymer aggregation. Although their trial should be highly valued, there were some problems in their samples: (i) the pyrenyl moiety was too bulky to neglect its hydrophobic interaction with another pyrenyl group for the polymer-polymer association in water, (ii) as the pyrenyl group in Winnik and co-workers' samples was far from the cellulose main chain (12 atoms spaced between the pyrenyl group and the anhydroglucose unit), it was difficult to represent the motion of the molecule, and (iii) as the fluorescence probes were randomly introduced into the cellulose polymer, it was hard to observe a very local change in a molecule.

In order to obtain more microscopic information on the aggregated state formed by the hydrogen bonds of cellulose, the benzyl group was chosen as one of the smallest fluorescent probes. Then the author prepared cellulose samples whose hydroxyl groups are regioselectively substituted by them and reported in a previous paper that the hydroxyl groups at the C-6 position of cellulose repeating unit plalys the most important role in their gelation process[13]. The benzyl fluorescence in a gel form was found to be different from that in a solution form, indicating that the fluorescence was influenced by the polymer-polymer association, since the fluorescence probes were situated in the vicinity of the anhydroglucose units.

In this chapter, the author prepared five kinds of cellulose samples, whose regioselective substitution patterns by benzyl ether and methyl ether groups were different from one another, and tried to clarify (1) the function of each hydroxyl group of cellulose having different preferences for forming a hydrogen bond, and (2) what causes the aggregation among the polymers. With the fluorescent-labelled cellulose, the temperature dependence of fluorescence behavior accompanying the gelation process will be reported.

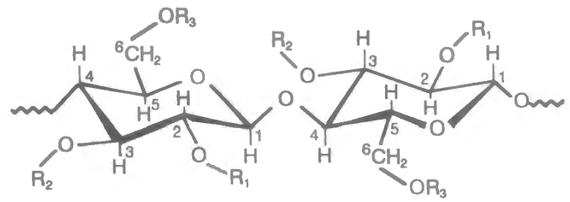
EXPERIMENTAL

Materials

The cellulose samples, whose hydroxyl groups were regioselectively substituted by benzyl ether and methyl ether groups, used in the present study were 6-*O*-benzylcellulose (6-BC), 2,3-di-*O*-methyl-6-*O*-benzylcellulose (2,3-M-6-BC), 2,3-di-*O*-benzylcellulose (2,3-BC), 2,3-di-*O*-benzyl-6-*O*-methylcellulose (2,3-B-6-MC), 2,3,6-tri-*O*-benzylcellulose (2,3,6-BC) (see Table 7-1). The details of the method to prepare 6-BC and 2,3-BC have already been reported[13]. The synthesis of the other cellulose derivatives was carried out as follows. Through the preparation of the samples, they were all arranged to have almost the same degree of polymerization (DP):*M*w of $4x10^4$.

All the products were dried under vacuum at 65°C and characterized by FT-IR and ^1H and ^{13}C NMR measuremens.

Table 7-1. Structure of the cellulose derivatives used for the measurements.



Sample		R_1	R_2	R_3
Cellulose		H	H	H
6-O-Benzylecellulose	(6-BC)	H	H	Benzyl
2,3-Di-O-methyl-6-O-benzylcellulose	(2,3-M-6-BC)	Methyl	Methyl	Benzyl
2,3-Di-O-Benzylcellulose	(2,3-BC)	Benzyl	Benzyl	H
2,3-Di-O-benzyl-6-O-methylcellulose	(2,3-B-6-MC)	Benzyl	Benzyl	Methyl
2,3,6-Tri-O-Benzylcellulose	(2,3,6-BC)	Benzyl	Benzyl	Benzyl

2,3-Di-*O*-methyl-6-*O*-benzylcellulose (2,3-M-6-BC): First, 2,3-di-*O*-methylcellulose (2,3-di-*O*-MC) was prepared by the method reported previously[14]. The free hydroxyl groups at the C-6 position were then benzylated in a dimethyl sulfoxide (DMSO) solution of the 2,3-di-*O*-MC, and the product was isolated and purified in the same manner as before[15]. The degree of substitution (DS) by benzyl groups was 1.0, while DS by methyl groups was 2.0.

2,3-Di-*O*-benzyl-6-*O*-methylcellulose (2,3-B-6-MC): Following the preparation of 2,3-BC[15], the latter was dissolved completely at 50°C in DMSO containing a trace amount of water. Methylation of the 2,3-BC was performed and then the 2,3-B-6-MC produced was isolated and purified according to a previous method[14, 16, 17]. The DS by benzyl groups was 2.0, while DS by methyl groups was 1.0.

Tri-*O*-benzylcellulose (2,3,6-BC): To prepare 2,3,6-BC, the author used 2,3-BC prepared above as a starting material to obtain the polymer with the same DP. It was dissolved completely in DMSO at 50°C and the same benzylation and isolation procedures for the above 2,3-M-6-BC were followed. The DS by benzyl groups was 3.0.

Analyses

Fluorescence spectra and fluorescence excitation spectra were measured on a Hitachi F-3000 spectrofluorometer. The emission signal was digitized and transferred into an NEC personal computer system. All the measurements were performed for aerated solutions of tetrahydrofuran (THF), which was purchased from Wako Co. (spectrograde). Fluorescence measurements for the concentrated THF solution of all the cellulose samples were carried out in a quartz cell with an optical pathlength of 1 mm. A cell was set at 45° to the exciting beam. The excitation wavelength was chosen to be 257 nm. The sample temperature was controlled by an Oxford DN1704 cryostat with an ITC-4 digital temperature controller. The temperature regulation was easily better than ± 0.1 K; independent temperature measurements were carried out using a second thermocouple and a potentiometer. All samples were kept at each set temperature, and spectra were obtained repeatedly for quite a long time (~100 h) even after perfect duplication was obtained, since one of the main aims of the present work is to determine the time required for the equilibrium.

RESULTS AND DISCUSSION

Gelation of the Cellulose Derivatives in THF

Concentrated THF solutions of the cellulose samples were quenched to several low temperatures in order to examine occurrence of the gelation after preparing isotropic solutions above 313K. Even the saturated (~3%) solutions of 6-BC, 2,3-M-6-BC, 2,3-B-6-MC and 2,3,6-BC were found to remain in solution state below 258K. In contrast, the gelation of 2,3-BC in THF was ensured by tilting the test-tube containing the solution[18]: the solution of 2,3-BC with a concentration higher than 1% (w/w) formed gel below 270K. These results provide two important suggestions: (1) the intermolecular

relationship between cellulose molecules examined requires the hydrogen bonds engaged in the OH groups at the C-6 position, while the OH groups at the the C-2 and C-3 position do not contribute to making aggregation, and (2) as is distinct from the case of aqueous solutions, the hydrophobic interaction between the bulky phenyl groups may not be attributed to the polymer-polymer association in a relatively polar organic solvent such as THF. The OH groups at the C-6 position is thought to be favorable for engaging the hydrogen bonds with other anhydroglucose units or other molecules, because it is a primary OH and the farthest group from the rigid polymer main chain and may be more mobile than other OH groups. Kondo and Sawatari[19] showed by FT-IR analysis of

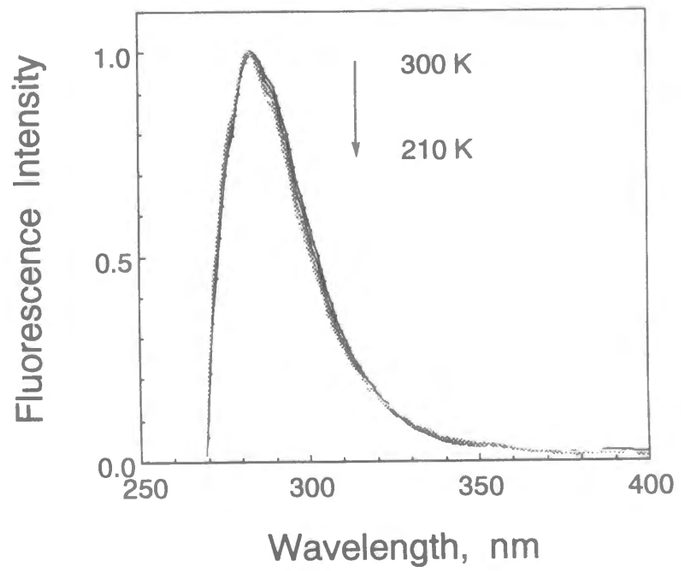


Figure 7-1. Temperature dependence of fluorescence spectra of 1.5% 2,3-M-6-BC in aerated THF in the cooling process from 300 to 210 K: the spectra are normalized at the peak (the excitation wavelength is 257 nm).

amorphous solid that the OH groups at the C-6 position may play a crucial role in determining the crystallization state for cellulose due to its high ability to form intermolecular hydrogen bonds.

Fluorescence Behavior of 2,3-M-6-BC, 6-BC, 2,3-B-6-MC and 2,3,6-BC in THF

Next, the author measured the fluorescence behavior of concentrated THF solutions of 6-BC, 2,3-M-6-BC, 2,3-BC, 2,3-B-6-MC and 2,3,6-BC in order to study the information on the microenvironment around phenyl groups.

Figure 7-1 shows the temperature dependence (from 300 to 210 K) of fluorescence spectra of 1.5wt% concentration of 2,3-M-6-BC in THF. All the spectra are normalized at the peak. The fluorescence spectra almost completely agree with one another and no other emission such as an excimer one appears. Each fluorescence spectrum is reproducible and identical at each temperature for both heating and cooling processes. The change of the fluorescence spectra of 2,3-M-6-BC at 210K for 30 h was also measured during the time course; however, all the spectra perfectly coincided with one another. Moreover it was ascertained that the temperature dependence of fluorescence spectra of 2,3-M-6-BC at a diluted concentration of 5×10^{-3} M for benzyl moiety in THF completely agreed with those of concentrated solutions. As a matter of course, the gelation did not take place in 1.5 wt% concentration of 2,3-M-6-BC in THF. Thus, Figure 7-1 shows the fluorescence behavior of an isolated benzyl group at the C-6 position even at such higher concentrations.

Figure 7-2 shows the temperature dependence (from 300 to 230K) of fluorescence spectra of 1.5 wt% concentration of 2,3-B-6-MC in THF. All the spectra normalized at the peak are identical with one another and almost agree with the spectra of 2,3-M-6-BC. It indicates that (1) there is no special interaction such as forming an excimer between the benzyl groups at the C-2 and C-3 positions and (2) the fluorescence behavior is almost the same among the benzyl ether groups at the C-2, C-3, and C-6 positions in the solution state.

The same results are obtained for the temperature dependence of 6-BC and 2,3,6-BC: no excimer fluorescence is detectable. In conclusion, no bimolecular process such

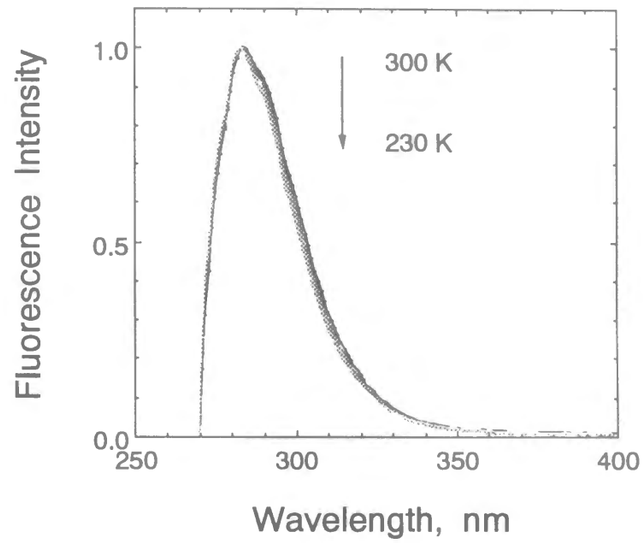


Figure 7-2. Temperature dependence of fluorescence spectra of 1.5% 2,3-B-6-MC in aerated THF in the cooling process from 300 to 230 K: the spectra are normalized at the peak (the excitation wavelength is 257 nm).

as excimer or dimer formation was observed in concentrated THF solutions of the cellulose derivatives which did not form gels.

Fluorescence Behavior of Concentrated THF Solution of 2,3-BC

Figure 7-3A shows the temperature dependence (from 300 to 200K) of fluorescence spectra of 2.3% 2,3-BC in THF. The spectra are found to change, apparently depending on temperature. In order to clarify the temperature dependence of 2,3-BC fluorescence, the author compared the shape of the fluorescence spectra normalized at each peak. The change for the cooling process can be divided into two temperature regions, i.e., (I) 305 - 270K (Figure 7-3B) and (II) 270 - 210K (Figure 7-3C). The change in region (I) was due to the red-shift of the fluorescence, while the change in region (II) was due to the formation of excimer. When the temperature of 2,3-BC in THF was raised to 300K,

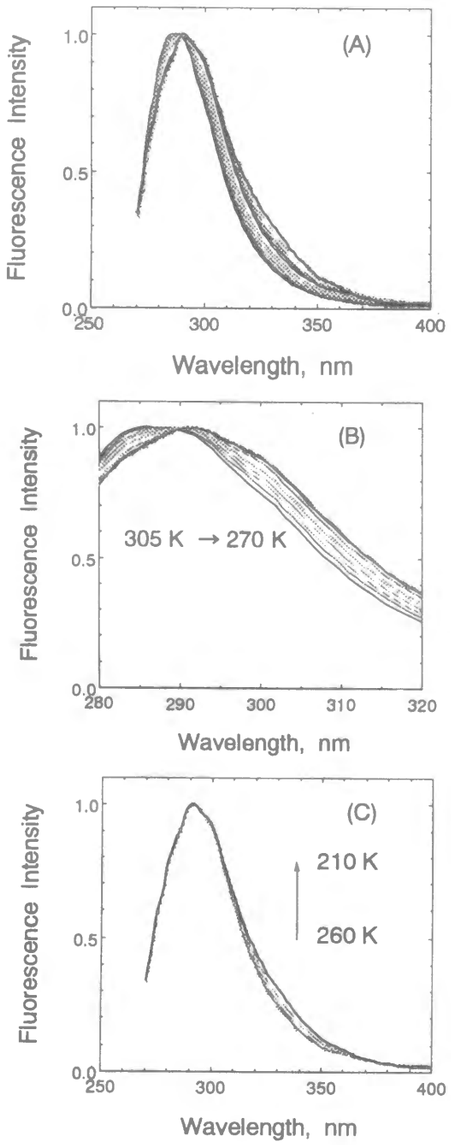


Figure 7-3. Temperature dependence of fluorescence spectra of 2.3% 2,3-BC in aerated THF in the cooling process from 300 to 200 K: (A)1, 305K; 2, 295K; 3, 283K; 4, 275K; 5, 270K; 6, 250K; 7, 230K; 8, 210K (excitation wavelength 257 nm). The spectra normalized at the peak are also shown separately in two figures: (B) 305 - 270K; (C) 270 - 210K.

the spectral shape that was shifted to the red at low temperatures returns to the shape before cooling.

The gelation of 2.3 wt% concentration of 2,3-BC in THF was ensured when the solution was cooled to 290K; thus, both the fluorescence changes (I) and (II) should reflect the change accompanied by the gelation process and/or the aggregation process.

As a matter of fact, it took about 2 weeks to obtain Figure 7-3 because it was ascertained whether the equilibrium was reached at each temperature. In the case of the gradual cooling process like this, the time required for the equilibrium at each temperature is not so long as the rapid quenching process: the equilibrium is reached within 10 h. However, the shape of the fluorescence spectra changes with time for 4 or 5 days in the case of rapid cooling from ~315K to lower temperatures such as 270K. The spectra change following the rapid quenching is identical with that shown in Figure 7-3, i.e. the red-shift of the fluorescence peak and the formation of excimer.

Figure 7-4 shows the excitation spectra for 320 nm fluorescence of 1wt% concentration of 2,3-BC in THF, when it is rapidly cooled from 315 to 273K. It shows that it takes nearly 4 days to attain the equilibrium state. The most important point shown in Figure 7-4 is that the red-shift of benzyl fluorescence is due to the formation of the new complex that is supposed to be a ground state dimer: the absorption near 280 nm appears and its intensity increases as time proceeds. This change coincides with the red-shift of the fluorescence shown in Figure 7-3B. The temperature dependence of the excitation spectra correponding to the fluorescence change shown in Figure 7-3 is identical with the change shown in Figure 7-4. The excitation wavelength was fixed at 257 nm; thus, the change in the fraction of the free benzyl group and the ground state dimer of the benzyl groups would give rise to the apparent red-shift of the fluorescence peak. Since a new emission was not observed in the concentrated solutions of 2,3-B-6-MC and 2,3,6-BC, the ground state dimer is considered to be formed intermolecularly.

The above results suggest that the extent of the aggregation can be estimated by the change in the fluorescence spectra. The fluorescence change can be expressed by the

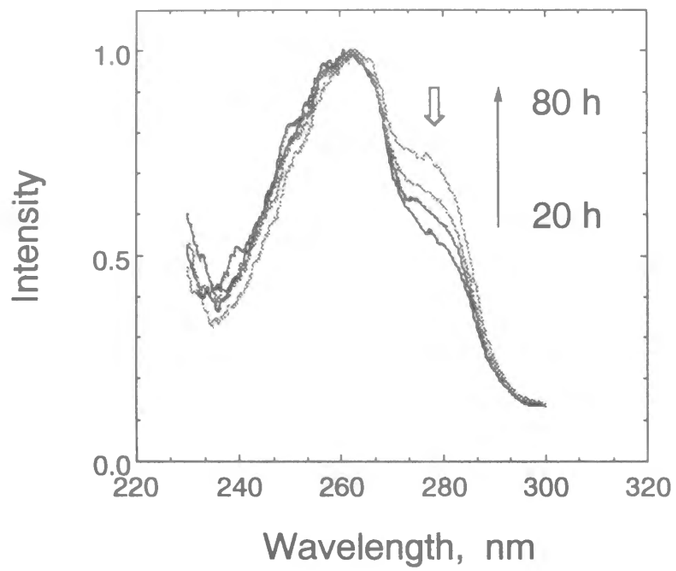


Figure 7-4. Fluorescence excitation spectra at 320 nm of 1% 2,3-BC in THF, when it is rapidly cooled from 315 to 273K. The arrow shows the new absorption band corresponding to ground state dimer.

intensity ratio of the emission at 330 nm, I_{330} to that at the peak, I_{peak}. When the degree of the polymer-polymer interaction increases, the I_{330} /I_{peak} increases because both the red-shift and the excimer formation make it larger. Figure 7-5 shows temperature dependence of I_{330} /$I_{peak}$of the 2.3 wt% concentration of 2,3-BC in THF, which is obtained by continuous measurements for 3 weeks from 305K to 200K and again from 200K to 310K. All the I_{330} /I_{peak}values are of the equilibrium state. Figure 7-5 demonstrates that a hysteresis loop is observed in the temperature dependence of fluorescence behavior of 2,3-BC. When the measurements were finished at 310K, 2,3-BC in THF was of the gel form and it was found to be melt to a transparent solution at 320K. Thus, all the data except a few initial points in the cooling process shown in Figure 7-5 were found to give information on a gel form of 2,3-BC in THF.

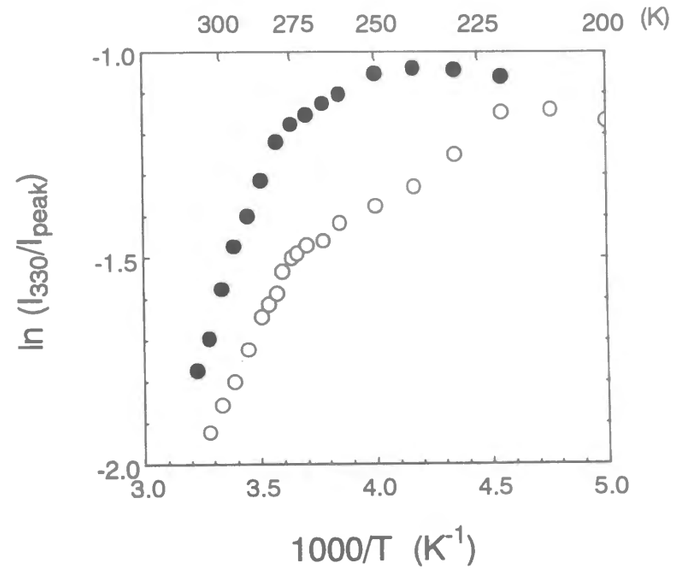


Figure 7-5. Temperature dependence of I_{330} /I_{peak} for 2.3% 2,3-BC in THF: (◯) cooling from 305 to 200 K; (●) heating from 200 to 310 K. All the I_{330} /I_{peak} values are the equilibrium ones.

The hysteresis loop observed in Figure 7-5 indicates that the cooling to 200K make the intermolecular interaction stronger at lower temperatures. The formation of the polymer network in 2,3-BC-THF attains equilibrium very quickly at each temperature; however, the number of cross-linking points of the gel network is shown to increase with lowering of the temperature. The present fluorescence probe method has first clarified that the change in the cellulose gel structure still proceeds at low temperatures. In general, the intermolecular hydrogen bond is not necessarily fixed at high temperatures where molecular motion is sufficiently fast. However, once the hydrogen bond is formed, it does not dissociate until the temperature is high enough to exceed the binding energy of the bond. Since lowering the temperature restricts the motion of groups such as OH groups at the C-6 position, the formation of a cross-linking point due to a hydrogen bond is considered to be very much encouraged at low temperatures.

Moreover the author would like to point out one important conclusion from the experimental results. It is the formation of the bimolecular complex supposed to be a ground state dimer, as shown in Figure 7-3B. The formation of an intermolecular dimer between the benzyl groups indicates the presence of a hydrophobic bond connecting two cellulose units. This dimer is not very stable, so that its fluorescence and absorption peaks diminish at temperatures higher than 300K. On the other hand, it is stable at low temperatures once the polymer-polymer interaction is formed by hydrogen bonding. Since 6-BC, 2,3-B-6-MC, 2,3-M-6-BC, and 2,3,6-BC samples in THF did not form gels and no dimeric fluorescence was observed, clearly the main cause for the gelation was not the hydrophobic effect between the bulky benzyl groups, but the formation of hyrogen bonds with the hydroxyl group at the C-6 position. It can be said that the fluorescence probe method has proved that hydrophobic bonding exists in the gel of 2,3-BC in THF, although its effect is minor in the formation of gels.

CONCLUSIONS

The author has examined gelation in THF solutions of five different types of cellulose derivatives whose OH groups are regioselectively substituted by benzyl ether and methyl ether groups. It was found that the gelation does not take place in THF solutions of the cellulose derivatives whose OH groups at the C-6 position are substituted to methyl ether or benzyl ether groups; 2,3-M-6-BC, 6-BC, 2,3-B-6-MC and 2,3,6-BC. These samples showed only the usual fluorescence of benzyl group. On the other hand, the cellulose derivative with the free OH1 groups at the C-6 position, i.e., 2,3-BC, was found to form gels in THF. Accompanying the gelation, the fluorescence of 2,3-BC shifted to the red and the excimer fluorescence increased. The new absorption corresponding to the red-shifted fluorescence peak appeared. This new species was concluded to be a ground state dimer formed intermolecularly between benzyl groups.

In conclusion, the hydrogen bond engaged in the OH groups at the C-6 position of cellulose is the major interaction forming a cross-linking point of the cellulose gel network in THF. The present fluorescence probe method has clarified first that the

change in structure of cellulosic gels still proceeds at low temperatures. At lower temperatures, the molecular motion of the OH groups at the C-6 position was restricted very much; thus, it may be concluded that the number of the hydrogen bond increases with lowering of the temperature.

Although the main cause of gel formation in 2,3-BC-THF system is the formation of the intermolecular hydrogen bonds, the fluorescence behavior also elucidated that the hydrophobic interaction between benzyl groups keeps 2,3-BC molecules associated with one another.

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Part III

Application of Regioselectively Substituted Cellulose Derivatives

Chapter 8

Enzymatic Degradation of Regioselectively Substituted *O*-Methylcelluloses

ABSTRACT: Regioselectively substituted methylcelluloses having a uniform structure, 6-*O*-methylcellulose **1**, was degraded by cellulase of *Trichoderma viride* while 2,3-di-*O*-methylcellulose **2** was not. In contrast, substrate **3** having a trace amount of unsubstituted units in compound **2** was depolymerized by the cellulase, suggesting that glycosidic bonds not only between anhydroglucose units regioselectively substituted at the C-6 position, but also between non-substituted and 2,3-di-*O*-substituted units were cleaved by the cellulase.

INTRODUCTION

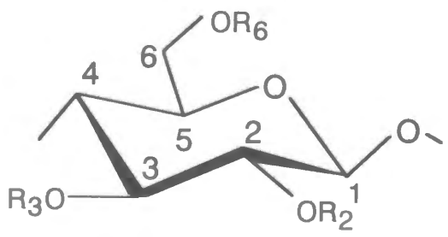
Cellulose and cellulose derivatives are known to be degraded by cellulase. The enzymatic degradation is considered as an ecologically important system that extends a possibility to apply biodegradable materials and therefore has been studied extensively. While the glycosidic linkages of cellulose are completely cleaved to yield glucoses, in the enzymatic hydrolysis of the cellulose derivatives such as *O*-methylcellulose, *O*-carboxymethylcellulose and *O*-hydroxyethylcellulose, it has been so far reported that the chain scission is assumed to mainly occur between two or more adjacent unsubstituted anhydroglucose (AHG) units, and can also occur at a residue having a 6- or 2-substituted residue as an aglycone[1-3]. Those studies were primarily based on the investigation of the structure of the produced AHG units by the enzymatic degradation. Namely, as compounds obtained by the treatment were glucoses and oligomers all having at least an unsubstituted AHG unit, it is assumed to

be essential to have an unsubstituted AHG unit in the hydrolysis by cellulase. However, this is not an enough evidence for the assumption. Because the substrates thus far used were not homogeneously substituted within an AHG unit, the structure of the neighboring AHG unit to the hydrolyzed oligomer was uncertain. In addition, because of the low yield of the scission between the unsubstituted and substituted units in heterogeneously substituted cellulose derivatives, it was difficult to detect the reaction itself. Therefore, it is necessary to have a substrate of cellulose derivatives having a uniform structure for investigating the structure of the scission point clearly and easily.

In Chapters 2 and 3, the author synthesized regioselectively substituted *O*-methylcelluloses having a uniform structure[4,5]. These polymers enable us to evaluate the cellulase-degradation of homogeneous cellulose derivatives. In this chapter, by the examination of the enzamatic degradation of the cellulosic homopolymers it would be shown that the glycosidic linkage between the adjacent AHG units thus particularly substituted as well as that among unsubstituted AHG units can be cleaved by the cellulase.

EXPERIMENTAL

Materials



	R_2	R_3	R_6
1:	H	H	CH_3
2:	CH_3	CH_3	H

Figure 8-1. The substrates used for the enzymatic hydrolyses in this chapter.

O-Methylcelluloses, MC3000P (Daiichi Kogyo Seiyaku. Co.) and MC 400CP (Wako Chem. Co.) were employed as commercial *O*-methylcellulose substrates. 6-O-methylcellulose **1** and a substrate **3** having a trace amount of unsubstituted units in compound **2** were synthesized according to the methods described in previous chapters (Chapters 2 and 3)[4,5]. Substrates **1** had no unsubstituted AHG unit and the unsubstituted unit of **3** was 3 %, determined by gas chromatographic analysis of alditol acetates of the acid-hydrolyzates.

Meicelase (CEPB-5081: Meiji Seika) originated from *Trichoderma viride* was used as a cellulase.

Analyses

All substrates were analyzed by ^{13}C-NMR spectroscopy for identification. The concentration for a substrate was 0.29 mg/ml for **1** and 0.98 mg/ml for others in 50 mM sodium acetate beffer (pH 5.0) and 1.0 % of the cellulase to the amount of the substrate was added. The solution was incubated at 37 °C. A reaction mixture was sampled after a certain period of the incubation. The extracted sample was measured by a size exclusion chromatography (SEC) with a HPLC system (TOSOH Co.) to observe the change of distribution of the molecular weight. The column for the SEC was TSK gel G2500 PWXL (Tosoh; 7.8 mm ID x 30 cm) and the eluent was 50mM sodium acetate buffer (pH 5.0). The average molecular weight (MW) of the products was estimated on the basis of a pullulan calibration. The degraded products were also provided for the determination of the produced reducing sugar-end by Somogyi-Nelson method[6] with 30 % dimethyl sulfoxide as solvent for dissolving all fractions from high to low molecular weight products.

RESULTS AND DISCUSSION

SEC chromatograms in Figure 8-2 clearly show the changes of distribution of molecular weights and decrease of the average molecular weights of (**1**) and (**3**) after

90 hours' cellulase treatment (broken line in (a) and (b), respectively). The increase of reducing ends produced from (**1**) and (**3**) also supports the depolymerization by the

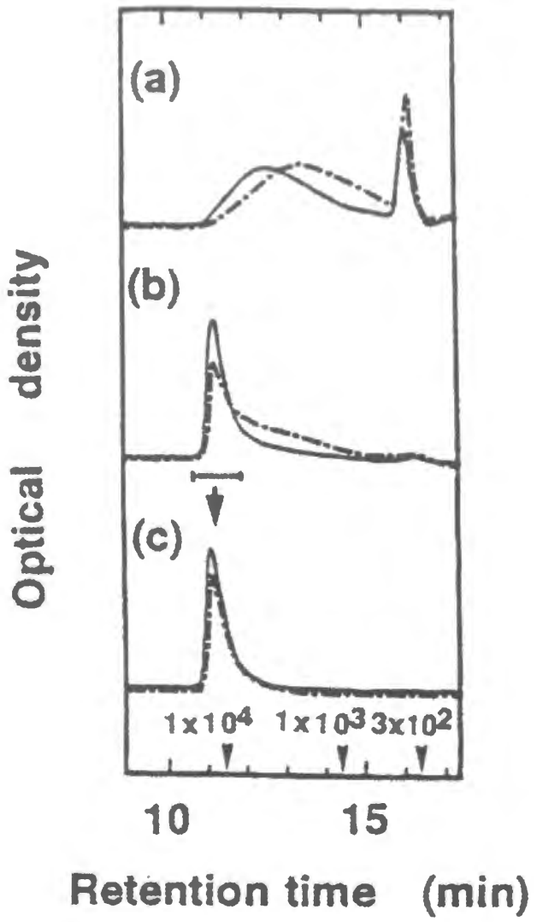


Figure 8-2. Size exclusion chromatograms of *O*-methylcellulose substrates before and after cellulase treatment. (a) **1**, (b) **3**, and (c) **2**. Solid and broken lines are untreated and after 90 hours' treatment, respectively. Numbers in the figure show the MW corresponding to the retention time. The arrow in (b) indicates the fractionated portion for (c).

enzymatic hydrolysis (Figure 8-3). After 24 and 96 hours' cellulase treatment, the amounts of the reducing ends produced in substrate (**1**) were 3.5 % and 5.1 %, respectively. The enzymatic hydrolysis of (**1**) further proceeded slowly. As already mentioned, compound (**1**) had a uniform structure and no unsubstituted AHG unit. Thus, these results indicate that the glycosidic linkage between the two 6-*O*-substituted AHG units can be cleaved. The enzymatic hydrolyzates were not monomeric sugars, but oligomers. The degree of polymerization of the oligomers was approximately 8 on the basis of MW. This suggests that endo-type cellulase predominantly hydrolyzed the substrate (**1**), and that exo-type cellulase and β-glycosidase considered to have a specificities to the terminal residue did not work because of the hindrance of the methyl substituent for the OH groups at the C-6 position of an AHG unit. The peak at about 16 min of the retention time was assumed to be dimer fractions including contaminants from the untreated substrate. In addition, an enzymatic reactivity to (**1**) was higher than that to commercial *O*-methylcelluloses as shown in Figure 8- 3.

Furthermore, in the SEC pattern (b) of Figure 8-2 for substrate (**3**), the low molecular weight fraction increased to reach 1.6 % with increasing ratio of the produced reducing ends after 96 hours' treatment. However, there appeared to still remain non-degraded high molecular weight fraction that was over $1x10^4$ in MW. As compound (**3**) was synthesized under a homogeneous reaction condition[5], the unsubstituted AHG units corresponding to 3 % was assumed to distribute at random along a cellulosic molecular chain. Therefore, the scission of glycosidic linkages by the cellulase could be considered that it occurred between the 2,3-di-*O*-substituted and non-substituted AHG units in stead of the cleavage between the two substituted units.

To confirm the postulation, as shown in Figure 8-2 (c), the apparently intact fraction with high molecular weight was fractionated repeatedly and purified to give ca. 2 in the Mw/Mn value. The obtained fraction was provided for ^{13}C-NMR measurement. Figure 8-4 shows the ^{13}C-NMR spectra in a region of C-1 carbon of **3**

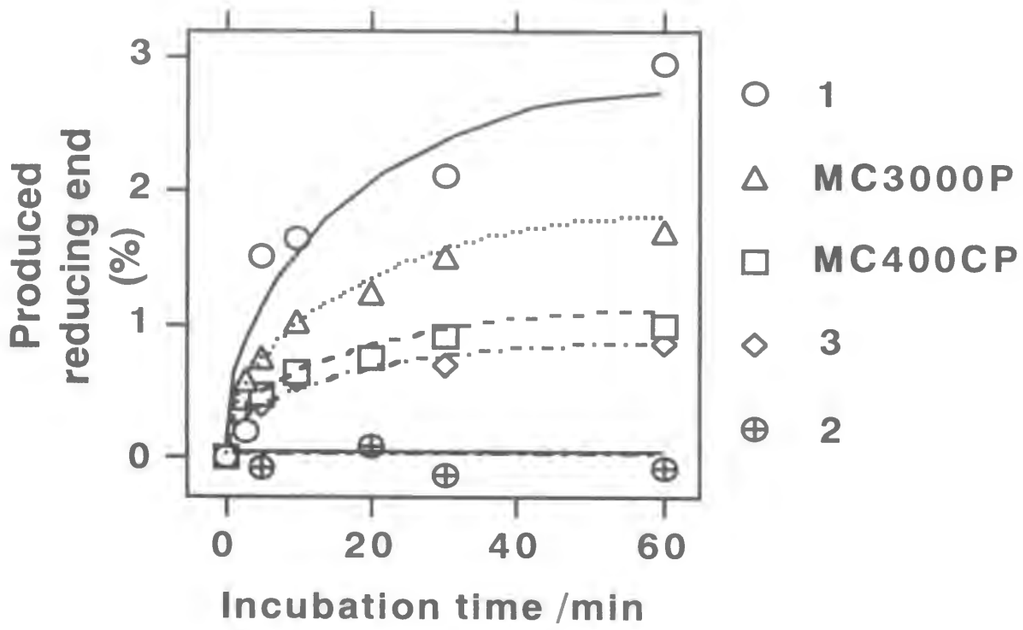


Figure 8-3. Changes of reducing ends produced from various *O*-methylcelluloses by the cellulase treatment.

$$\text{Produced reducing end (\%)} = \frac{\text{Number of produced reducing ends} \times 100}{\text{Number of glucosidic bonds of untreated substrate}}$$

and the fractionated product. Generally the presence of an ether substituent at the C-2 position causes an upfield shift of the C-1 resonance relative to that of glucose[7]. In the spectrum of **3**, a main signal was 101.1 ppm due to C-1 carbon of 2,3-di-*O*-substituted AHG unit of **2**.

Besides, there appeared two signals of 102.5 and 101.8 ppm due to C-1 carbon of unsubstituted AHG and non-identified one, respectively. After the fractionation, the two signals disappeared and only a main signal corresponding to the same one of **3** appeared. Consequently, the fractionated compound was identified as completely methylated fraction at the C-2 and C-3 positions in **2**. As shown in Figure 8-2(c) and

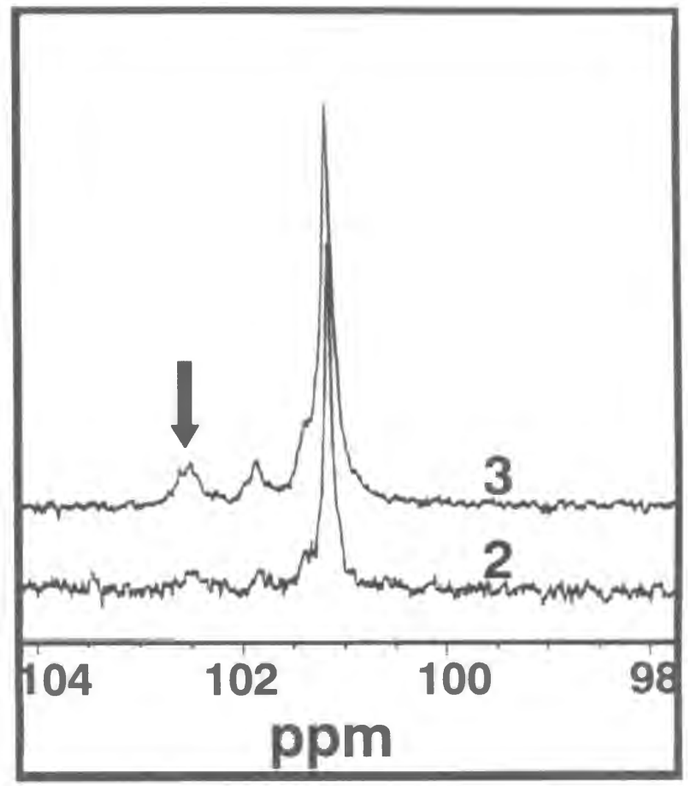


Figure 8-4. ^{13}C-NMR spectra with a range of C-1 carbon of the AHG unit of compunds **3** and **2**. The arrow indicates C-1 carbon signal of the unsubstituted AHG unit.

Figure 8-3, the substrate **2** was intact before and after the cellulase treatment, suggesting that the cellulase has no activity against **2**. These results show that while the cleavage of the glycosidic bond between the two AHG units in compound **2** was completely inhibited by the substitution at the C-2 and C-3 positions, the scission of the bond occurred when the adjacent AHG unit to the 2,3-substituted unit was unsubstituted.

There seems to be some inhibiting factors on the substrate **2**. An effect of the molecular structure is among them. The catalytic mechanism of cleavage of glycosyl oxygen by cellulase has been explained as an analogy of hydrolysis by lysozyme[8]: It split glycosidic linkages by a mechanism of an acid catalysis with participation of carboxyl group of glutamic acid residue (Glu) and aspartic acid residue (Asp). Considering intramolecular hydrogen bonds in **1** and **2**[9], the glycosidic oxygens in **1** appear to be exposed to the aqueous media. On the other hand, the oxygens in **2** are covered in a hydrophobic atmosphere by the substitution at the C-2 and C-3 positions. Therefore, the hydrophobic atmosphere in **2** may inhibit the approach of amino acids such as Glu and Asp in the active center of cellulase to the glycosyl oxygen. In addition, Legler and Bause reported that there are two or three binding subsites adjacent to the catalytic site towards the nonreducing end[10]. In substrate **2**, there may not have a binding subsite because of its hydrophobicity. On the contrary, the 6-*O*-methyl substituent in **1** is rather at a distance from the main chain. Thus, we assume that the hydrophobic inhibition in **1** has less influence than that in **2** and endo-type cellulase can hydrolyze the glycosidic bonds between the two 6-*O*-substituted AHG units. The kinetics of the enzymatic reaction, the effect of the primary and secondary structures of the AHG unit and the identification of the active cellulase by purification of the crude cellulase are now required.

CONCLUSION

In summary, it was found that cellulose derivatives can be hydrolyzed by cellulase when the primary structure of the AHG unit is controlled by regioselective substitution. Namely, glycosidic bonds not only between anhydroglucose units regioselectively substituted at the C-6 position, but also between non-substituted and 2,3-di-*O*-substituted units were cleaved by the cellulase, which may provide an important information for further developments of biodegradable materials from cellulose derivatives.

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Summary

In Chapter 1, a simple way to prepare tri-*O*-alkylcelluloses from cellulose acetate in dimethylsulfoxide (DMSO) using the appropriate alkyl iodide and NaOH was developed. In this procedure, the addition of a small amount of water to the DMSO solution improved the efficiency of etherification. This etherification method can be applied to the alkylation of other polymers with acetate-functional groups that are soluble in DMSO.

In Chapter 2, *O*-methyl and *O*-ethylcelluloses having controlled distribution of substituents were prepared by alkylation of 6-*O*-triphenylmethyl ("trityl") cellulose in DMSO and subsequent detritylation of the alkylated products. Water-free alkylation reactions failed to give complete substitution of the OH groups at both the C-2 and C-3 positions. The addition of a small amount of water to the tritylcellulose solution in DMSO improved the efficiency of the alkylations, yielding 2,3-di-*O*-substituted-6-*O*-tritylcellulose, which in turn gave the 2,3-di-*O*-alkylcelluloses on detritylation with HCl gas. Repeated further alkylation of the 2,3-di-*O*-alkylcelluloses in DMSO furnished products with high degrees of substitution at the C-6 position. The distribution of methyl and ethyl groups in the polymers prepared by repeated alkylation systematically changed with each alkylation step.

In Chapter 3, regioselectively substituted at the primary OH groups, 6-*O*-alkylcelluloses, were prepared from tritylcellulose. In the preferred procedure, the tritylcellulose was first completely allylated in DMSO, and subsequently detritylated with hydrogen chloride to yield 2,3-di-*O*-allylcellulose. This product was isomerized into 2,3-di-*O*-(1-propenyl)cellulose with potassium *tert*-butoxide in DMSO. The polymer thus prepared was then alkylated completely with methyl or ethyl iodide, in DMSO containing a trace of water. The alkylated polymer was finally treated with 0.1M HCl in aqueous 90 methanol at room temperature to remove the 1-propenyl groups. The products were shown by Fouriertransform infrared spectroscopy (FT-IR), ^{13}C-nuclear magnetic resonance spectroscopy (NMR), and gas-chromatographic analysis (GLC) to be uniformly substituted at the C-6 position.

In Chapter 4, formation of hydrogen bonds in regioselectively substituted cellulose derivatives prepared, 2,3-di-*O*- and 6-*O*-substituted cellulose ethers, were characterized by FT-IR and CP/MAS ^{13}C-NMR spectroscopies. Since their three OH groups were selectively blocked, the cellulose derivatives appeared to form specific inter- and intramolecular hydrogen bonds. The characteristic OH stretching frequencies in IR spectra and the C-1 chemical shift in CP/MAS spectra of 6-*O*-substituted cellulose derivatives provided a possibility of existence of two almost equivalent intramolecular hydrogen bonds between ether oxygen and OH groups at 3-OH---O5' and O6---HO-2'. Behavior of the hydrogen bonds in 6-*O*-triphenylmethylcellulose ("tritylcellulose") was also discussed.

In Chapter 5, some direct evidences about the relationship between the intramolecular hydrogen bonds in cellulose and their corresponding effects on physicochemical properties were provided. The formation of intramolecular hydrogen bonds was proved to contribute directly and indirectly to certain physicochemical properties of cellulose, such as its solubility in solvents having different polarities, the relative reactivities of the hydroxyls in a repeating unit, and its crystallinity, using a 6-*O*-methylcellulose (6-*O*-MC) film.

In Chapter 6, it was shown that intramolecular hydrogen bonds formed in the OH groups at the C-3 position of the anhydroglucose units contributed to the determination of the handedness of chiral nematic mesophases *O*-ethylcellulose in CH_2Cl_2. Using two series of samples which differed only by increasing the ethyl substitution of the OH groups at either the C-6 or C-3 positions, the handedness of individual mesophase solutions was investigated using circular dichroism (CD). The CD spectra indicated that free OH groups at the C-6 position hinder dissolution in the solvent while OH groups at the C-3 position may play an important role in determining the handedness of the EC mesophase in CH_2Cl_2. Free OH groups at the C-3 position are known to easily

form intramolecular hydrogen bonds, resulting in enhanced stiffness of the molecular chains. Therefore, these results lead to the conclusion that the breaking of intramolecular hydrogen bonds by increasing the substitution along the molecular chain causes it to become more flexible, which may account for the change in structural handedness.

In Chapter 7, five cellulose derivatives whose OH groups were regioselectively substituted by benzyl ether and methyl ether groups were preprared; i.e., 6-*O*-benzylcellulose, 2,3-di-*O*-methyl-6-*O*-benzylcellulose, 2,3-di-*O*-benzylcellulose (2,3-BC), 2,3-di-*O*-benzyl-6-*O*-methylcellulose, 2,3,6-tri-*O*-benzylcellulose. The gelation did not take place in tetrahydrofuran (THF) solutions of the cellulose derivatives whose OH groups at the C-6 position were substituted to methyl ether or benzyl ether groups, but the derivative having free OH groups at the C-6 position, i.e., 2,3-BC, was found to form gels in THF. The present method using fluorescent probe was found to be very useful to elucidate the gelation process with a change of temperature in terms of the molecular association involving a hydrogen bond. In conclusion, (1) the main cause for the gel formation in the systems examined is the hydrogen bonding due to the OH groups at the C-6 position and (2) in addition to hydrogen bonding, the hydrophobic interaction between benzyl groups also keeps 2,3-BC molecules associated with one another after it is aggregated.

In Chapter 8, regioselectively substituted methylcelluloses having a uniform structure, 6-*O*-methylcellulose, was degraded by cellulase of *Trichoderma viride* while 2,3-di-*O*-methylcellulose was not. In contrast, 2,3-di-*O*-methylcellulose having a trace amount of unsubstituted units was depolymerized by the cellulase, suggesting that glycosidic bonds not only between anhydroglucose units regioselectively substituted at the C-6 position, but also between non-substituted and 2,3-di-*O*-substituted units were cleaved by the cellulase.

As concluding comments from the author, the following will be presented: Cellulose comprises the major polymer of plant cell walls and has had a long history as

a natural polymer material which has been used maily in textile and paper industries. In this sense, cellulose is a conventional and old polymer compared with other newly synthesized polymers in recent years. However, as mentioned in General Introduction, cellulose exhibits really versatile characteristics thanks to its unique self-organization manners. The major reason for the self-organization seems to be due to hydrogen bonding engagements as well as van der Waals force. In other words, if we could control the hydrogen bonding manners, it would enable us to produce other kinds of new biodegradable materials from cellulose molecules. This possibility should be also added to the versatility. Therefore, we should review this promising material from a totally different standpoint from the previous. The author attempted in this thesis to collect information on the formation of the hydrogen bonds as much as possible in order to open the above possibility using regioselectively substituted cellulose derivatives. The compounds themselves also exhibited very interesting phenomena that may extend to another usage of cellulose derivatives even though they are bearing the same substituent and the same degree of substitution (DS). It should be noted that the substitution is spreading regioselectively and homogeneously throughout the molecular chain.

Another important point for the investigation carried out in this thesis was whether the cellulosic material was amorphous or crystalline. To date people have been paying more attention to native crystalline cellulose or crystalline regions. However, when considered for the usage, it should be considered whether the cellulosic material is highly crystalline, low crystalline or amorphous. In other words, it is of importance to obtain the information not only on the crystal structure, but also on the non-crystalline structure. The author wants to insist this point. We should pay more attention to non-crystalline regions of cellulose than the present. Using regioselectively substituted cellulose derivatives, it would also be possible to obtain some information on the structure in the non-crystalline regions of cellulose.

Finally, the author hopes that the regioselective modification methods and the products reported in this thesis could open further versatility for cellulosic materials.

List of Publications

Chapter 1.

Facile Method for the Preparation of Tri-*O*-(Alkyl) Cellulose;

T. Kondo and D. G. Gray

J. Appl. Polym. Sci., **45**, 417-423 (1992).

Chapter 2.

The Preparation of *O*-Methyl- and *O*-Ethyl-celluloses Having Controlled Distribution of Substituents;

T. Kondo and D. G. Gray

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Chapter 3.

Preparation of 6-*O*-Alkylcelluloses;

T. Kondo

Carbohydr. Res., **238**, 231-240 (1993).

Chapter 4.

Hydrogen Bonds in Regioselectively Substituted Cellulose Derivatives;

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Chapter 5.

The Relationship between Intramolecular Hydrogen Bonds and Certain Physical Properties of Regioselectively Substituted Cellulose Derivatives;

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Chapter 6.

The Influence of Intramolecular Hydrogen Bonds on Handedness in Ethylcellulose /CH_2Cl_2 Liquid Crystalline Mesophases;

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Chapter 7.

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Chapter 8.

Characterization of the Cleavage of β-Glucosidic Linkage by *Trichoderma viride* Cellulase Using Regioselectively Substituted Methylcelluloses;

T. Kondo and M. Nojiri

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1) Order Parameters and Side-Chain Conformation in Ethylcellulose/ Chloroform Liquid Crystal Phases;

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H. Itagaki, I. Takahashi, M. Natsume and T. Kondo
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Tetsuo Kondo